ASSESSMENT REPORT ON

SULPHUR DIOXIDE

FOR DEVELOPING AMBIENT AIR QUALITY OBJECTIVES

EFFECTS ON VEGETATION

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AMBIENT AIR QUALITY OBJECTIVES

- Effects on Vegetation

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PREFACE

A multi-stakeholder workshop was held in October 2000 to obtain input from stakeholders on priorities for development or review of ambient air quality guidelines (AAQG). Workshop participants identified the sulphur dioxide ambient air quality guideline as one of the existing guidelines that required review. This document presents sulphur dioxide effects on vegetation and is one of two science assessment documents for sulphur dioxide.

The purpose of this document is to summarize selected primary literature on the effects of SO_2 exposure on vegetation. This document is not intended to be a comprehensive review of the published literature. Instead an attempt was made to select work that would provide relevant information for reviewing the existing ambient air quality objective (AAQO) for SO_2 for Alberta. Basic criteria for selecting studies included:

- Ambient and/or experimental SO_2 concentrations were provided
- The use of realistic SO_2 exposure concentrations
- Species native (or similar) to ones grown in Alberta
- Measurements of vegetation effects were quantifiable and repeatable

Part I provides an over view of studies conducted prior to 1990. Although an attempt was made to select studies that used realistic concentrations of SO_2, many of the early studies discussed in Part I used high SO_2 concentrations (many exceeded 1,000 $\mu g\ m^{-3}$). However, as the understanding of sulphur dioxide effects on plants advanced, the concentrations of SO_2 used in research were generally reduced to more realistic levels, many that might be observed in the environment. Part II presents literature from 1990 to 2002, in which the majority of the studies used more realistic concentrations of SO_2.

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LIST OF ABBREVIATIONS

AAQO	Ambient air quality objective(s)
AAQG	Ambient air quality guideline(s)
ABA	Abscisic acid
ATP	Adenosine triphosphate
avg	Average
CF	Charcoal filtered
CO_2	Carbon dioxide
Cu	Copper
cv	Cultivar
d	Day(s)
DW	Dry weight
FBPase	Fructose-1,6-bisphosphatase
FeSOD	Iron superoxide dismutase
FC	Fusicoccin
g	Gram
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate-pyruvate transaminase
>	Greater than
GSH	Reduced glutathione
h	Hour(s)
HCl	Hydrogen chloride or hydrochloric acid
HSO_3^-	Hydrogen sulphite ion (bisulphite)
IAP	Index of atmospheric purity
K	Potassium
km	Kilometre
kPa	Kilopascals
<	Less than
L	Litre
m	Meter
min	Minute(s)
$\mu g\ m^{-3}$	Micrograms per cubic meter
mon	Month(s)
N	Nitrogen
$Na_2S_2O_5$	Sodium hydrogensulfite
NAAQO	National Ambient Air Quality Objectives

NADP-GPD	NADP-dependent glyceraldehyde-phosphate dehydrogenase
NF	Non-filtered
Ni	Nickel
NO_2	Nitrogen dioxide
O_3	Ozone
P	Phosphorous
PES	Energy used for photosynthesis
PGK	Phosphoglycerate kinase
PMCs	Pollen mother cells
PPB	Parts per billion
PPM	Parts per million
PRK	Phosphoribulokinase
RH	Relative humidity
RuDPC	Ribulose-diphosphate carboxylase
S	Sulphur
SO_2	Sulphur dioxide
SO_3^{2-}	Sulphite ion
SO_4^{2-}	Sulphate anion
SOD	Superoxide dismutase
VAM	Vesicular arbuscular mychorrizae
wk	Week(s)
y	Year(s)

1.0 INTRODUCTION

Sulphur (S) is one of the essential elements plants require for growth and reproduction. Plant roots can absorb sulphate anions (SO_4^{2-}) from the soil; alternatively, leaves can absorb sulphur dioxide (SO_2) from the atmosphere. When soil S is inadequate, atmospheric SO_2 can be used as an alternate S source. However, uptake of excess S from the soil or the atmosphere, or a combination of the two can result in injury to vegetation.

When exposed to air pollutants the amount of pollutant that enters the plant has the greatest effect on the degree of injury. In addition, many biotic and abiotic factors play a role in the degree of damage when plants are exposed to SO_2. Biotic factors include growth stage, genetic make-up, plant nutrient status, insects, and disease. Abiotic factors include light, moisture availability, temperature, relative humidity, and the presence of other air pollutants.

The major route of atmospheric gases, including SO_2, into plants is through the stomata (apertures in the leaf epidermis, which can open and close to control gas exchange). When exposed to SO_2, some plants close the stomates thus reducing SO_2 uptake, but not all plants close their stomates upon exposure (Black, 1985). Water stress can also result in reduced stomatal opening, which would result in reduced SO_2 uptake. In order to enter through open stomata, SO_2 must first diffuse across a layer of "unstirred" air surrounding the leaf called the boundary layer. Increased air movement can decrease the diffusive resistance of the boundary layer (Salisbury and Ross, 1992) resulting in increased SO_2 uptake.

After absorption, SO_2 dissolves in the aqueous phase of the cell wall to form bisulphite (HSO_3^-) or sulphite (SO_3^{2-}), which then undergoes enzymatic conversion to SO_4^{2-}. The SO_4^{2-} is then transported into leaf cells where it is incorporated into organic molecules, including amino acids such as cysteine and methionine, which are then incorporated into proteins (Rennenberg and Herschbach, 1996). When excess SO_2 enters the plant the resulting injury may:

- become visible as foliar injury (e.g. necrotic or dead tissue, chlorosis)
- result in disruption of metabolic or physiological processes, which lead to growth and yield reductions
- result in accumulation of excess S in plant tissues.

Sulphur dioxide injury to vegetation is characterized as being a result of either acute or chronic exposure and is generally thought of as follows (Legge *et al.*, 1998):

- Acute exposures are a few minutes to a few hours at concentrations that result in visible injury (chlorosis or necrosis of tissue) within a few hours or days.
- Chronic exposures are long-term (weeks to years), to low concentrations that result in metabolic injury and may or may not result in visible injury.

The concentrations of SO_2 in the literature were reported using various units, for example: parts per billion (ppb), parts per million (ppm), or micrograms per meter cubed ($\mu g\ m^{-3}$). To facilitate comparison between studies all concentrations were converted to $\mu g\ m^{-3}$, assuming standard temperature (25°C) and pressure (101.3 kPa).

The report has been divided into sections based on the effects of sulphur dioxide on different aspects of plant physiology (e.g. sulphur content, visible injury, growth); however, many of the studies examined more than one of these possible effects. For ease of reading, each study is included in only one section of the report.

PART I

2.0 LITERATURE PRIOR TO 1990

Physiological changes in plants in response to exposure to SO_2 can involve from a few cells to the whole plant. These changes can include: reduced photosynthetic activity, increased respiration, alteration of enzyme production, changed growth (increased or decreased), and/or altered uptake and accumulation of nutrients (including S). The extent of change is a result of the concentration of SO_2 and exposure duration, the relative sensitivity of the plant to SO_2, and what mechanisms the plant can employ to detoxify SO_2, or the ability of the plant to repair damage that has occurred due to exposure. The following studies describe the effects that can occur as a result of exposure to SO_2.

2.1 Effects on Growth

Cowling and Lockyer (1976) studied the effects of SO_2 exposure on the growth and sulphur nutrition of perennial ryegrass (*Lolium perenne* L.). They compared the growth of plants exposed for 87 d, in chambers, to a daily average of 50 $\mu g\ m^{-3}$ SO_2 or filtered air, with or without adequate soil sulphur. Plants grown without additional soil S exhibited S deficiency symptoms and growth was reduced. Exposure to SO_2 increased the root and shoot dry weight of plants grown in sulphate deficient soil, but did not restore growth to levels observed with adequate soil S. However, SO_2 exposure did not impact the shoot growth of plants grown in sulphate-sufficient soil. Shoot sulphur declined in all treatments throughout the exposure period, but exposure to SO_2 lessened the decline. It was also observed that exposure to SO_2 resulted in an increase of shoot sulphate-S as a proportion of the total S. It was indicated that although SO_2 was used as a S supply, in these experiments only the soil applied sulphate provided adequate sulphur (Table 1).

In a further study on how nutrient supply influences the effect of SO_2 on perennial ryegrass, Cowling and Lockyer (1978) exposed plants for 85 d, in chambers, to a daily average of 55 $\mu g\ m^{-3}$ SO_2 or filtered air. Plants were grown with or without added soil sulphur and two rates of added nitrogen (N: low and high). Plants grown with high N and no added soil S developed S deficiency symptoms. The S deficiency symptoms were reduced in the high N treatment in the presence of SO_2 while shoot dry weight more than doubled and root dry weight almost doubled. When high N plants were supplied with soil sulphate, the addition of SO_2 to the atmosphere did not alter root or shoot dry weight. The number of tillers increased with increased N, with added soil sulphate, and when exposed to SO_2. There was little difference in shoot or root dry weights and number of tillers in the low N treatments in the presence or absence of SO_2. Total shoot sulphur and sulphate-S generally increased with the addition of soil sulphate and exposure to SO_2; although, high N treatments had lower levels of both which was attributed to dilution, as the high N plants were larger (Table 1).

The effects of SO_2 exposure on red pine (*Pinus resinosa* Alt) seedlings in the cotyledon stage were examined to determine possible effects on seedling establishment (Constantinidou *et al.*, 1976). Seedlings were harvested 11 weeks after exposure to 1,310, 2,619, 7,857 or 10,476 $\mu g\ m^{-3}$ SO_2 in Plexiglas exposure cubes for 15, 30, 60 or 120 minutes. Exposure of cotyledons resulted in reduced chlorophyll content which decreased as exposure concentration or time increased. Cotyledon dry weight also decreased as exposure concentration and time increased, with significant differences from controls in the highest two concentrations for the 60 and 120 minute exposures. Analysis indicated that exposure time was more important than concentration for chlorophyll and dry weight of cotyledons. Primary needles exhibited the same pattern of response as the cotyledons (chlorophyll content and dry weight decreased as exposure time or concentration increased). In addition, emergence of primary needles appeared to be inhibited but the effect was not significant. Visible injury was apparent within 1 day of fumigation in 15% (7,857 $\mu g\ m^{-3}$ SO_2) and 25% (10,476 $\mu g\ m^{-3}$ SO_2) of seedlings when exposed for 60 or 120 minutes (Table 1).

Ashenden and Mansfield (1977) recognized that air movement could influence SO_2 uptake into plants so they investigated the influence of wind speed on the effects of SO_2 on perennial ryegrass (*Lolium perenne* L.). Plants were exposed in wind tunnels to 288 $\mu g\ m^{-3}$ SO_2 at two wind speeds, 10 and 25 m min^{-1}. Each experiment was conducted twice, and day and night temperatures varied slightly between the experiments, which altered plant growth but did not change the pattern of response. When exposed to SO_2 with a wind speed of 25 m min^{-1} there were significant reductions in leaf area, root/shoot ratio, and dry weight of: green leaf material, dead leaves + stubble, total shoot, and roots. In contrast, dry weights of plants exposed with a wind speed of 10 m min^{-1} were not significantly reduced but there was a significant increase in the number of fully expanded green leaves. The authors concluded that the greater sensitivity to SO_2 exhibited at the higher wind speed was probably a result of reduced boundary layer resistance of the leaf (Table 1).

Roberts (1975) compared the growth of seedlings of SO_2-sensitive white birch (*Betula papyrifera* Marsh) and SO_2-tolerant pin oak (*Quercus palustris*). Seedlings were grown in pots (sunk into the ground and mulched), in the field, where they were exposed for three months to SO_2 levels of <10 (ambient) or 70.8 $\mu g\ m^{-3}$ (annual averages). Plants were harvested biweekly from mid-June until the end of August. Dry weight of white birch leaves, stems, and roots showed no significant differences until the mid-August harvest, at which time there was a significant increase in leaf and stem weights of plants in high SO_2 compared to low SO_2. Final height of the birch seedlings was also significantly greater in the high SO_2 plots. The author thought that the increase in growth of birch was due to the additional S supply. In contrast, pin oak height was significantly less and dry weight was reduced in the high SO_2 compared to the low SO_2 treatment. It was suggested that the reduction in growth of pin oak was probably due to stomatal closure (a common resistance mechanism observed in SO_2 resistant species) in response to high ambient SO_2 exposure, which resulted in reduced photosynthesis (Table 1).

2.2 Sulphur Dioxide Uptake and Plant Sulphur Content

When exposed to SO_2 there can be wide variation in the absorption of and accumulation of sulphur compounds between plant species. The following studies describe some of the differences that have been observed.

In a study on the capacity of alfalfa (*Medicago sativa* L.) to neutralize and metabolize absorbed SO_2, Thomas *et al.* (1944) found that exposures of 628 to 338 h, to 492 or 762 $\mu g\ m^{-3}$ SO_2 respectively, did not change the pH of leaf extracts but did reduce their buffering capacity. The plants were able to neutralize the absorbed acid, which is one mechanism of SO_2 resistance. It was also found that the absorbed SO_2 was transformed principally to sulphate and small amounts of organic S compounds. In addition, alfalfa and sugar beets (*Beta vulgaris*) were exposed to 487 $\mu g\ m^{-3}$ SO_2 for 5.2 h d^{-1}, for 60 days, while being grown under various nutrient regimes. When the nutrient solution was deficient in sulphate, fumigated leaves had higher levels of labile organic sulphur than the non-fumigated leaves. When sulphate was at sufficient levels in the nutrient solution there was little difference in labile S between treatments (Table 1).

The differences in SO_2 absorption of four cultivars of cucumber (*Cucurbitaceae*) were studied after exposure to SO_2 (1,048 to 23,571 $\mu g\ m^{-3}$ for 360 to 1,430 minutes) (Bressan *et al.*, 1977). Sulphur dioxide concentrations used in this study were much higher than would be expected to occur at a polluted site but the authors were able to show that the most resistant cultivars absorbed the least SO_2. Differential absorption between the cultivars could not be attributed to stomatal behaviour, as it was not examined in this study. It was also observed that young leaves were more resistant than old leaves but there was no difference in SO_2 absorption between the two. It was concluded that a biochemical mechanism conferred greater resistance to the younger leaves.

Miller and Xerikos (1979) treated 'sensitive' and 'tolerant' cultivars of soybean (*Glycine max* L. Merr.) in 0.08 M sodium sulphite (Na_2SO_3) solutions (equivalent to 10 mg ml^{-1}, or 26,190 $\mu g\ m^{-3}$ SO_2) for 30 minutes to determine if the residence time of sulphite in plant cells relates to varietal or species resistance. The 'resistant' cultivars had consistently less sulphite at the end of the treatment period than 'sensitive' cultivars despite taking up more of the treatment solution. It was suggested that the reduction of sulphite levels was a result of conversion to sulphate and that 'resistant' cultivars metabolized sulphite more efficiently than 'sensitive' cultivars. A further test indicated that after a 30-minute treatment period, leaves of 'resistant' cultivars contained significantly less of the absorbed sulphite, 4.9%, compared to 11.5% in 'sensitive' cultivars.

2.3 Visible Injury

Symptoms of SO_2 injury can be described as either acute or chronic. Acute injury results from short-term exposures to high concentrations that result in cellular death of all or part of the plant (Legg *et al.*, 1998). Chronic injury is the response to long-term exposures to sub-lethal concentrations that result in alterations in cellular metabolism and may or may not exhibit visible injury (Legge *et al.*, 1998). The following studies investigated the effects of both acute and chronic exposures to SO_2 on the appearance of visible injury symptoms.

Katz and McCallum (1939) conducted a series of field plot experiments to determine the relative sensitivity of a number of conifer species to SO_2. The experiments used a number of SO_2 concentrations up to 13,933 $\mu g\ m^{-3}$ with exposure times of a few hours to 69 d. The appearance of injury symptoms was influenced by a number of factors and varied by species and SO_2 exposure (see Table 1). No visible injury was observed on any of the exposed trees at 576 $\mu g\ m^{-3}$ for up to a 69 d exposure (Oct 8 to Dec 18). Injury was delayed when exposures were conducted in late fall (Nov 23 to Dec 2). The observed delay in onset of injury was attributed to lowered plant activity as the temperature decreased. Relative humidity (RH) was shown to be one of the factors affecting injury as injury to western larch (*Larix occidentalis*) appeared in 8 h at 786 $\mu g\ m^{-3}$ (May 29) at 67% RH but injury did not appear in a 76.5 h exposure up to 1,100 $\mu g\ m^{-3}$ (May 29) at 45% RH. The conifers were ranked for their susceptibility to SO_2 using the degree of injury sustained along with the length of time for initial injury symptoms to appear. The species were ranked (in order of susceptibility) as follows: larch, Douglas fir, Engelmann spruce, white pine, yellow pine, cedar, lodgepole pine, silver fir and white fir. In addition, total needle sulphur content was determined in four of the experiments and results indicated that up to the time of incipient injury the rate of SO_2 absorption was influenced more by the concentration of SO_2 than the time of exposure (Table 1).

The results of a five year study by Linzon (1966) indicated that the deterioration of eastern white pine (*Pinus strobes* L.), in permanent plots to the northeast of Sudbury, correlated with the increase in atmospheric SO_2 as the distance to the source (smelter) decreased. When the distance to the smelter exceeded 25 miles and ambient SO_2 levels were consistently below 655 $\mu g\ m^{-3}$ the health of the pines had not deteriorated. Linzon concluded that SO_2 higher than 655 $\mu g\ m^{-3}$ for several hours could damage foliage of eastern white pine.

O'Connor *et al.* (1974) tested 131 native Australian plant species to determine their sensitivity to acute SO_2 exposure. Plants were exposed to 800 $\mu g\ m^{-3}$ for 27 h, 2,620 $\mu g\ m^{-3}$ for 3 and 6 h, 5,240 $\mu g\ m^{-3}$ for 4 h, or 7,860 $\mu g\ m^{-3}$ for 0.5, 1, 2, 4 and 6 h. The measure of sensitivity used was the percent of necrotic leaf tissue that had developed 3 or 4 days after fumigation. It was observed that most species showed increased sensitivity with increased concentrations of SO_2 or with increased exposure times. Also, "injury occurred most rapidly and extensively to fully-developed actively-photosynthesising leaves" while young expanding leaves were more resistant. It was also noted that sensitivity of species within genera could be highly variable in their response to acute exposure to SO_2.

Taylor *et al.* (1986) investigated physiological differences of two ecotypes of geranium (*Geranium carolinianum*) that differed in their sensitivity to SO_2. Although SO_2 uptake was equivalent between sensitive and resistant ecotypes, leaf necrosis (as % of the total leaf area) for the sensitive ecotype averaged 31.1% while the resistant ecotype averaged only 4.6% after 5 h exposure to 2,095 $\mu g\ m^{-3}$ SO_2. Photosynthesis was measured every hour for 5 hours during exposure to 786 to 1,571 $\mu g\ m^{-3}$ SO_2. Inhibition of photosynthesis in the resistant ecotype was dependent on concentration but independent of time. From 3 to 5 h exposure the percentage inhibition increased from an average of 11.5% at 786 $\mu g\ m^{-3}$ to 29.9% at 1,571 $\mu g\ m^{-3}$ SO_2. In contrast, photosynthesis in the sensitive ecotype was inhibited at all concentrations of SO_2, 786 $\mu g\ m^{-3}$ and higher and the percentage inhibition increased significantly with exposure time. Measurement of efflux of hydrogen sulphide (H_2S) (one of the mechanisms plants use for removal of excess S) indicated the rate of efflux increased with SO_2 concentration but did not differ significantly between the ecotypes. Long-term exposure to 1,179 $\mu g\ m^{-3}$ SO_2 for 6 h d^{-1}, 4 d wk^{-1}, for 4 weeks resulted in a 28% increase (resistant ecotype) and 26% decrease (sensitive ecotype) in photosynthesis from controls. In addition, photoassimilate retention in the foliage was reduced 6% in the resistant ecotype and increased 7% in the sensitive ecotype. Average shoot or root dry weight of the sensitive ecotype was 10 to 25% lower than the resistant ecotype at equivalent SO_2 concentrations and exposures. The results suggested the genetic differences between the ecotypes resulted in physiological expression of different biochemical threshold levels of response to SO_2, or homeostatic processes governing repair and compensation for injury (Table 1).

2.4 Other Metabolic Processes

Exposure of plants to SO_2 can result in disruption of normal metabolic activity. Enzymatic activity can be increased or decreased or photosynthesis and respiration can be altered. The degree of metabolic change is dependent on the concentration of SO_2 and the length of exposure. It is possible that metabolic disturbance can occur without visible signs of injury. Following are studies that indicate the broad range of metabolic response that may be observed when plants are exposed to SO_2.

Horsman and Wellburn (1977) used broad-leafed dock (*Rumex obtusifolius*) collected from areas of high ambient (annual mean 150 $\mu g\ m^{-3}$) and low ambient (30 $\mu g\ m^{-3}$) SO_2 to determine effects of SO_2 exposure on enzyme activities. Plants were fumigated with 524 $\mu g\ m^{-3}$ SO_2 for 11 days in a wind-tunnel fumigation system. No visible injury was observed on any of the plants, and leaf fresh weights were not affected. Ribulose-diphosphate carboxylase (RuDPC) in low ambient area leaves was reduced to 65% (young) and 68% (old) of controls. Fumigated high ambient plants exhibited little change in RuDPC in either young (97%) or old leaves (109%). Peroxidase levels in the low ambient plants increased following exposure (140% young leaves, 135% old leaves). The high ambient area plants had significantly higher levels of peroxidase (>140%) than low ambient plants under control conditions and experienced relatively small changes in peroxidase following SO_2 exposure (114% young leaves, 105% old leaves). Glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) levels increased in fumigated young leaves (138% and 127% low ambient; 119% and 123% high

ambient), from both areas, while old leaves were not affected. It was concluded that the differences in enzyme activities between low and high ambient plants could be a result of development of sulphite tolerance in the high ambient plants and could be the reason for maintenance of RuDPC activity. The high activity of peroxidase in high ambient plants could reflect adaptation to the SO_2 levels of their natural environment (Table 1).

Harvey and Legge (1979) studied the effects of SO_2 on adenosine triphosphate (ATP) metabolism in lodgepole-jack pine (*Pinus contorta x banksiana*) hybrid foliage. Hybrid seedlings were grown under controlled chamber conditions then exposed in Siemens cuvettes to 200 to 800 $\mu g\ m^{-3}$ SO_2 for 0.5 h. In addition, needles were collected from trees, growing around a natural gas processing plant near Whitecourt, Alberta, during SO_2 fumigations. Sulphur dioxide levels at the field site were normally 0 to 656 $\mu g\ m^{-3}$ with the maximum level of SO_2 measured during this experiment being 542 $\mu g\ m^{-3}$. It was found that ATP in needles of field samples decreased linearly as SO_2 increased. Branches collected from the field site and used for controlled fumigations in the laboratory showed a similar response. In contrast, the previously unexposed foliage grown in growth chambers did not normally show a reduction in ATP during fumigation. It was concluded that the difference in response could be explained by adaptation of the field grown trees to low-level SO_2 fumigation (Table 1).

Tanaka and Sugahara (1980) used poplar (*Populus euramericana*) and spinach (*Spinacia oleracea* L.) cv. New Asia to show that increased resistance to SO_2 can be correlated with high levels of superoxide dismutase (SOD) activity in leaves. Plants were exposed in fumigation chambers to the SO_2 concentrations and times indicated below. They observed that young poplar leaves had up to five times more SOD activity than old ones when exposed to 5,238 $\mu g\ m^{-3}$ SO_2 for 22 hours. The higher SOD activities resulted in reduced chlorophyll destruction and lower malondialdehyde formation (a product of lipid peroxidation). In order to confirm the role of SOD in reducing SO_2 toxicity, diethyldithiocarbamate (DDTC, a SOD inhibitor) was applied to spinach leaves. This resulted in 65% reduction in SOD activities after 2 h exposure. Exposure of spinach leaves that had been treated with DDTC to 1,310 $\mu g\ m^{-3}$ SO_2 for 22 h resulted in enhanced chlorophyll destruction over untreated leaves. In addition, it was shown that pre-treatment of poplar leaves with 262 $\mu g\ m^{-3}$ SO_2 for 20 days increased SOD activities to 4.4 times the control. The pre-treatment resulted in a decrease in chlorophyll destruction when exposed to 5,238 $\mu g\ m^{-3}$ SO_2 for up to 24 h (Table 1).

Shimazaki *et al.* (1980) presented further evidence that SOD plays a role in SO_2 resistance. Photosynthetic pigments and lipids of spinach (*Spinacia oleracea* L.) cv. New Asia were studied in a series of experiments to determine the role of active oxygen species in SO_2 toxicity. Whole plants were exposed in fumigation chambers to 5,238 $\mu g\ m^{-3}$ SO_2 for the indicated times. Visible injury appeared around leaf veins 2-3 h after start of fumigation, in the light, and injured areas were completely white in 10 – 15 h; whereas, no injury occurred when leaves were exposed for up to 10 h in the dark. After 2 h exposure to SO_2, chlorophyll *a* and carotenoids were destroyed in the light but not in the dark. Anaerobic conditions reduced pigment destruction. When SOD was added to leaf reaction mixtures after 3 h fumigation, the destruction of chlorophyll *a* was reduced. After 2 h SO_2 fumigation the formation of malondialdehyde (a product of lipid peroxidation) was inhibited by the addition of scavengers of superoxide radicals (O_2^-)(e.g. hydroquinone, tiron, or ascorbate). These results indicated that the destruction of

chlorophyll *a* required both light and oxygen. It was concluded that lipid peroxidation in fumigated leaves resulted from the action of singlet oxygen produced from 0_2^- (Table 1).

Peiser *et al.* (1982) used ethane formation to test if lipid peroxidation in chloroplasts is induced by free radicals from sulphite oxidation. Isolated spinach chloroplasts (*Spinacia oleracea* L.) were incubated with solutions containing sulphate or sulphite and lipid peroxidation was measured by ethane production. In comparison to sulphate, it was found that sulphite produced approximately a 10-fold increase in ethane production in intact chloroplasts. They also observed that light was important for sulphite induced ethane production as little ethane was produced in the dark with or without sulphite. Ethane production was not inhibited in the presence of a scavenger of singlet oxygen but was inhibited by SOD, once again suggesting the involvement of 0_2^- in lipid peroxidation. While this study cannot be compared directly with gaseous exposures, it does provide support for the role of SOD in protecting against oxidative stress resulting from sulphite oxidation.

Olszyk and Tingey (1984a) studied the effects of light on the phytotoxicity of SO_2 using garden pea (*Pisum sativum* L.) cv Alsweet and a tomato mutant (*Lycopersicon esculentum flacca* Mill.). To ensure similar internal SO_2 exposure in dark and light exposures, the peas were sprayed with fusicoccin (FC), which induces opening of stomata in both light and dark. The stomata of the tomato mutant do not close in the dark due to a deficiency in abscisic acid (ABA). Pea plants were exposed, in fumigation chambers, to 1,310 to 2,619 $\mu g\ m^{-3}$ SO_2 and tomato plants to 1,571 to 2,095 $\mu g\ m^{-3}$ SO_2 for 2 hours, in the dark and in the light. Exposure in the light resulted in up to 80% (pea) or 64% (tomato) less leaf injury compared to exposure in the dark and the reduction in injury was most prominent in the oldest leaves. It was concluded that light-activated metabolism may have detoxified SO_2 itself, or repaired the SO_2 injury. It was also noted that these results are only applicable to acute exposures as plants may respond differently to chronic exposures (Table 1).

Further experiments were performed using garden pea (*Pisum sativum* L.) cv Alsweet and a tomato mutant (*Lycopersicon esculentum flacca* Mill.) in an attempt to determine the possible role of cellular transport processes in SO_2 toxicity (Olszyk and Tingey, 1984b). Plants were sprayed with fusicoccin (FC) to ensure stomata were open during fumigation. Peas were exposed to 1,833, 2,357 and 2,619 $\mu g\ m^{-3}$ SO_2 and tomato to 1,571 $\mu g\ m^{-3}$ SO_2 for 2 hours, in the light. When tomato was exposed to SO_2, FC treated plants had an average 21% necrotic leaf area; whereas, plants not treated with FC had significantly less injury, only 3% of the leaf area. Exposure to SO_2 of FC treated peas resulted in 2 to 8 times more leaf injury than untreated plants. The results of these experiments led the researchers to conclude that in addition to ensuring the stomata were open, FC stimulated the movement of SO_2 or one of its metabolites across the plasma membrane, which resulted in the enhancement of SO_2 injury (Table 1).

2.5 Effects on Pollen

As many studies had shown that exposure to SO_2 damaged foliage, reduced growth, and caused metabolic and enzymatic changes, many researchers believed it was important to evaluate the effects of SO_2 on reproduction. This section describes studies that examined the effects of SO_2 on pollen germination, pollen tube growth, and pollen chromosomal abnormalities after SO_2 exposure.

Ma *et al.* (1973) used spiderwort (*Tradescantia paludosa* Anders. Sax clone-3) to determine if exposure to SO_2 would increase the number of chromosomal aberrations in exposed pollen. Pollen was exposed in sealed Plexiglas containers to 131 to 262 $\mu g\ m^{-3}$ SO_2 from 5 to 10 minutes after sowing to the metaphase stage of mitosis (18 – 20 h). It was found that SO_2 enhanced the chromatid aberration rate under their experimental conditions. The average increase in aberrations was 46.4 breaks per 100 cells during summer experiments and 11.7 breaks per 100 cells in fall and winter experiments. The difference in aberration rates from summer to winter was attributed to higher light intensities in summer. It was suggested that SO_2 might interfere with the repair of damage such as enhanced sister chromatid exchange or lesions in chromosomes, which can be caused by photochemical reactions (Table 1).

Further experiments by Ma and Khan (1976) were conducted to determine if there was a dosage effect on pollen tube development and mitotic activity of the generative nucleus of spiderwort (*Tradescantia paludosa* Anders. Sax clone-3). Results indicated that mitotic activity of the generative nucleus was inhibited by exposure to 196 to 130,950 $\mu g\ m^{-3}$ SO_2 for 18.5 h in sealed Plexiglas chambers. Mitotic activity decreased linearly as the SO_2 concentration increased. Pollen tube growth was also measured after exposure to 26,190 to 5,238,000 $\mu g\ m^{-3}$ SO_2 for 18.5 h. It was found that there was no reduction in growth from 196 to 26,190 $\mu g\ m^{-3}$ SO_2, but from 130,950 to 2,619,000 $\mu g\ m^{-3}$ SO_2 pollen tube length was reduced from an average of approximately 880 to 140 μm. It was suggested that the response was saturated at 2,619,000 $\mu g\ m^{-3}$ SO_2 as there were no further growth reductions at the highest SO_2 concentration. It was concluded that the results of this and the previous study (Ma *et al.*, 1973) indicated that SO_2 damaged sites that are related to DNA metabolism (Table 1).

In vitro studies were used to determine if SO_2 exposure inhibited pollen germination and pollen tube growth of cottonwood (*Populus deltoides* Bartr.), Red pine (*Pinus resinosa* Ait.), Austrian pine (*Pinus nigra* Arnold), and blue spruce (*Picea pungens* Engelm.) (Karnosky and Stairs, 1974). Wet or dry pollen was exposed to 786 to 26,190 $\mu g\ m^{-3}$ SO_2 for 4 h. Pollen of cottonwood dehydrated and did not germinate after dry exposure; as a result, only wet exposure results were reported. Significant reductions in cottonwood pollen germination and pollen tube elongation were observed at or above 1,960 $\mu g\ m^{-3}$ and 786 $\mu g\ m^{-3}$ SO_2, respectively. When exposed dry, concentrations up to 26,190 $\mu g\ m^{-3}$ SO_2 had no effect on germination and tube elongation of the conifer pollen. In contrast, when wet, exposure to 3,667 $\mu g\ m^{-3}$ SO_2 resulted in greatly reduced germination and tube elongation (to a level < 20% of controls). It was suggested that the inhibition of pollen germination and tube elongation in cottonwood could have resulted from acidification of the germination media resulting from SO_2 absorption. For conifer pollen it

appeared that the form of absorbed SO_2 in the media had affected germination and elongation rather than the degree of acidification (Table 1).

Varshney and Varshney (1981) exposed chick pea, (*Cicer arietinum* L.); nasturtium (*Nasturtium indicum* L.); petunia, (*Petunia alba* Juss.); and spiderwort, (*Tradescantia axillaris* L.) pollen to a range of SO_2 concentrations when dry (1,310 to 13,100 $\mu g\ m^{-3}$), or wet (26.2 to 2,620 $\mu g\ m^{-3}$) for 1 to 5 h, in sealed chambers. Germination of pollen under moist exposure conditions was almost completely inhibited at 262 $\mu g\ m^{-3}$ SO_2 for the four species (1 to 3% of controls); whereas, dry exposure was not affected up to 7,860 $\mu g\ m^{-3}$ SO_2. It was suggested that the greater toxicity of SO_2 to moist pollen was due to its conversion to sulphite (SO_3^{2-}) or hydrogen sulphite (HSO_3^-) ions or a dissolved or unreacted form of SO_2. Moist exposure to SO_2 also greatly reduced pollen tube growth. There were reductions in length of 5.5 to 28.5% after 1 h exposure to 26.2 $\mu g\ m^{-3}$ SO_2, and 91.3 to 96.6% after 1 h exposure to 262 $\mu g\ m^{-3}$ SO_2. Pollen fumigation under dry conditions resulted in approximately 35% reduction in pollen tube growth for all species after 5 h exposure to 13,100 $\mu g\ m^{-3}$ SO_2. It was suggested that the reduction in growth was due to SO_2 effects on the generative nucleus (Table 1).

A number of in vitro studies demonstrated that SO_2 has a detrimental effect on pollen germination and growth. In order to determine the specific stage of sexual reproduction that was affected by SO_2, DuBay and Murdy (1983) conducted whole plant studies with geranium (*Geranium carolinianum* L.). Plants were exposed to 1,571 $\mu g\ m^{-3}$ SO_2 for 8 h at 70, 80, or 90% relative humidity (RH). In the 90% RH treatment, seed set, mean number of pollen germinated on the stigma and percent germination were significantly lower than the control by 34, 42, and 34%, respectively. At 70 or 80% RH there was slight visible injury to the leaves but there were no effects of SO_2 on any of the measured parameters. It was concluded that at the highest RH, SO_2 dissolved into the moist surface of the stigma and pollen grains, and affected the ability of the pollen to germinate (Table 1).

Many of the experiments on the effects of SO_2 on pollen were performed with extremely high concentrations that are unlikely to be observed in the environment. Keller and Beda (1984) decided to evaluate the effects of lower SO_2 concentrations on pollen of mugo pine (*Pinus mugo* Turra), scotch pine (*Pinus sylvestris* L.), Austrian pine (*Pinus nigra* Arnold), and silver fir (*Abies alba* Mill.). Pollen was exposed, in chambers, to 65, 195 or 589 $\mu g\ m^{-3}$ SO_2 for 16 h (pines) and/or 24 h (pines and fir) then germinated on agar medium in "moist chambers". Germination of pollen decreased by more than 25% (pines, 16 h exposure) and 30% (fir, 24 h exposure) at 195 $\mu g\ m^{-3}$ SO_2 and increasing the length of exposure of pine pollen from 16 to 24 h did not alter the magnitude of the response. Complete inhibition of germination was observed at the highest SO_2 level for all four species. It was concluded that tree reproduction could be affected in the field (Table 1).

Linskens *et al.* (1985) exposed petunia (*Petunia hybrida* Vilm.) Clone W166H pollen in vitro to 52,380 $\mu g\ m^{-3}$ SO_2, generated by mixing hydrochloric acid (HCl) and sodium hydrogen sulphite ($Na_2S_2O_5$). Pollen germination and tube growth were measured after 2 and 3 hours incubation, respectively. There were significant reductions from the controls in both pollen germination (45%) and tube length (32%). The reductions were attributed to reductions in pH (acidification) of the medium (Table 1).

Experiments in which Monterey pine (*Pinus radiata*) pollen was exposed to 262 to 26,190 $\mu g\ m^{-3}$ SO_2 for 24 h resulted in noticeably reduced pollen germination and approximately 50% reduction in pollen tube growth at 2,619 $\mu g\ m^{-3}$ SO_2 (O'Connor *et al.*, 1987). Chromatography assays were also performed to determine the effects of SO_2 on the metabolites of early germination. It was found that percent activity was reduced by 24% (aspartate), 13% (glutamine), 10% (alanine), and 26% (γ-amino butyrate) at 262 $\mu g\ m^{-3}$ SO_2. In addition, the number of identifiable metabolites was decreased. It was concluded that SO_2 inhibited the enzymes that catalyzed the formation of the metabolites (Table 1).

Faba bean (*Vicia faba* V. Giza 2) plants were exposed to SO_2 concentrations of 13,095 to 654,750 $\mu g\ m^{-3}$ for 5 h or 13,095 $\mu g\ m^{-3}$ for 5 h d^{-1} for 1, 2 or 4 days to determine if there were cytogenetic or viability effects on pollen (Amer *et al.*, 1989). The number of non-viable pollen grains increased significantly as the concentration of SO_2 increased. The authors used chromosome fragmentation as the criterion for mutagenic potential. Statistical analysis of the results indicated the percentage of abnormal pollen mother cells (PMC's) increased significantly after exposure to 13,095 $\mu g\ m^{-3}$ SO_2 for 5 h and the percentage of abnormal cells increased as the concentration of SO_2 increased or as the period of fumigation increased (Table 1).

2.6 Multiple Pollutant (Interaction) Experiments

Exposure to a single pollutant results in damage and/or growth losses that can be predicted based on previous experiments. However, when exposed to more than one pollutant, the response of plants can be difficult to interpret as the pollutants may 'act against each other' resulting in less damage than would be predicted, or 'act together' resulting in greater damage than predicted, or have no interaction at all. For clarity in reading the following, interactions of multiple pollutants will be defined as:

- When growth is less than that predicted, **synergistic** interactions are implied.
- When growth is greater than predicted, **antagonistic** interactions are implied.

Houston (1974) used greenhouse fumigation chambers to expose tolerant and sensitive clones of eastern white pine (*Pinus strobus* L.) to SO_2 and ozone (O_3) singly, and in combination, to determine the minimum concentrations that would result in foliage injury. Plants were exposed to 65 to 1,179 $\mu g\ m^{-3}$ SO_2, 98 to 1,117 $\mu g\ m^{-3}$ O_3, or 65 $\mu g\ m^{-3}$ SO_2 plus 98 $\mu g\ m^{-3}$ O_3, for 6 hours. Sensitive clones showed reduced needle elongation and visible foliar injury at all concentrations of SO_2. Tolerant clones did not exhibit foliar injury until exposed to 131 $\mu g\ m^{-3}$ SO_2 and injury increased in severity as the concentration of SO_2 increased. Ozone and SO_2 in combination resulted in synergistic effects on growth and foliar injury for sensitive clones. However, tolerant clones had no foliar injury but needle growth was depressed. It was concluded that tolerant clones might have suffered physiological injury without visible symptoms (Table 1).

In an attempt to determine threshold concentrations for injury of trembling aspen (*Populus tremuloides* Michx.) clones to O_3 and SO_2, Karnosky (1976) exposed plants for 3 h to 524 to 1,702 $\mu g\ m^{-3}$ SO_2, 98 to 392 $\mu g\ m^{-3}$ O_3, and performed two interaction experiments with concentrations of 524 or 916 $\mu g\ m^{-3}$ SO_2 plus 98 $\mu g\ m^{-3}$ O_3. The response of the 5 clones varied, 3 of the 5 clones showed injury to SO_2 at 916 $\mu g\ m^{-3}$ with injury generally increasing as SO_2 concentration increased. All five clones exhibited injury symptoms at 1,702 $\mu g\ m^{-3}$ SO_2. In the interaction experiments exposure to the combination of pollutants resulted in more of the clones and a greater percentage of the foliage being injured than when exposed to a single pollutant.

Experiments were conducted in which O_3 sensitive and non-sensitive loblolly pine (*Pinus taeda*) seedlings were exposed in fumigation chambers to O_3 (98 $\mu g\ m^{-3}$), SO_2 (367$\mu g\ m^{-3}$) or NO_2 (188 $\mu g\ m^{-3}$) singly, and in combination 6 h per day for 28 days. Experiments were to determine if ambient concentrations of the pollutants would cause foliar injury or affect growth (Kress *et al.*, 1982). The results indicated that the pollutants generally caused greater foliar injury and growth suppression when exposed in combination than when exposed singly. The authors also noted that when exposed to $O_3 + SO_2$ or $O_3 + SO_2 + NO_2$ the non-sensitive trees exhibited >10% height reductions and the sensitive trees exhibited significant height reductions (>18%). The height reductions were not always correlated with foliar injury. Less than 4% of the foliage of the sensitive family exhibited injury symptoms and the non-sensitive trees exhibited less injury than the sensitive trees in all treatments (Table 1).

Long-term exposure of Kentucky bluegrass (*Poa pratensis* L.) cv. Monopoly to 177 $\mu g\ m^{-3}$ SO_2 and 127 $\mu g\ m^{-3}$ NO_2 singly, and in combination was examined to determine how the response would change over an extended period (Whitmore and Mansfield, 1983). Seedlings were exposed from 0900 h Monday to 1700 h Friday each week, from emergence on October 26 to May 7. Six harvests were made at intervals between these dates. A significant interaction of $SO_2 + NO_2$ on dry weight was observed by the 2nd harvest (February) while individual effects of the two pollutants did not become apparent until the 4th harvest (April). At the final harvest, dry weight was reduced to about 55% of controls in the SO_2 treatment and to about 26% in the $SO_2 + NO_2$ treatment. A significant increase in the shoot:root ratio was observed in the SO_2 treatment. A similar experiment was conducted the following year, with the same pollutant treatments and measurements continuing until late summer. The effects on dry weight and shoot:rhoot ratio were generally the same as the previous experiment up until the May harvest; however, as this was the last harvest in which it was feasible to separate the roots subsequent data was only for shoots. Shoot growth recovered over the growing season and by the final harvest the SO_2 and $SO_2 + NO_2$ treatments showed a slight stimulation of growth. In contrast, both the SO_2 and $SO_2 + NO_2$ treatments, delayed the start of stem elongation and severely reduced the number of flowering heads at the final harvest. The authors concluded that the observed reductions in growth could be attributed to lower net assimilation rates while the increased shoot:root ratio was a compensatory mechanism to counteract reduced photosynthetic activity. It was also indicated that the increased growth observed over the summer in the second experiment was associated with reduced flowering and that reduced flowering may have been a result of delayed development (smaller plants during culm development in the spring) in the SO_2 and $SO_2 + NO_2$ treatments (Table 1).

A chamberless field exposure system was used to determine if there would be yield effects from exposure of soybean to SO_2 (26, 144 and 288 $\mu g\ m^{-3}$) and O_3 (82, 122 and 157 $\mu g\ m^{-3}$) singly, and in all combinations for 5.25 h d^{-1} for 16 of 23 days (Reich and Amundson, 1984). Fumigations were not conducted on days in which wind would greatly alter pollutant concentrations from designed levels. When exposed singly, O_3 significantly decreased all yield parameters measured; whereas, SO_2 only significantly reduced mass per seed by 4 to 7%. However, a non-significant seed yield reduction of 7% per plant and 12% per hectare occurred in the high SO_2 treatment (288 $\mu g\ m^{-3}$). No significant interactions were observed between SO_2 and O_3 and there were no visible signs of leaf injury in any treatments (Table 1).

Heggestad *et al.* (1986) investigated the impact of increasing SO_2 concentrations (13 to 1,226 $\mu g\ m^{-3}$) in the presence (non-filtered; NF), and absence (charcoal-filtered; CF) of ambient ozone. Tomato (*Lycopersicon esculentum*) cv Jet star plants were exposed, in open-top chambers, for 5 h d^{-1} (daylight), 5 d wk^{-1} for 57 days, July to September. Leaf injury increased, as the season progressed with significantly greater injury in the NF air indicating other pollutants, probably O_3 were a more important cause of injury than SO_2 alone. Increased SO_2 had no significant effects on plant height; however, plant and fruit weights decreased linearly with increasing SO_2. The ambient O_3 treatment and all non-filtered SO_2 treatments reduced the weight of ripe fruit. Statistical analysis indicated there were no interactive effects of exposure to the SO_2 and O_3. Increased SO_2 resulted in a linear increase in foliar S in both NF and CF treatments but did not change fruit S levels. There was no consistent change in the other foliar elements tested (Table 1).

2.7 Lichens

The study of physiological responses of lichens to pollutants is complex since each member (the fungus and the algae) play a specific role in the symbiosis. The algal portion of lichens occupies only 5-10% by weight (Farrar, 1973) but through photosynthesis fixes carbon and passes the carbon to the fungal portion in the form of simple carbohydrates. As lichens do not have roots, they depend on the atmosphere for nutrients and have developed nutrient concentrating mechanisms that are required for survival (Nash, 1996). These mechanisms have led to the use of lichens as monitors of atmospheric deposition. This section summarizes a few of the studies on lichen diversity and physiological responses to SO_2.

2.7.1 Lichen Diversity in Response to Sulphur Dioxide

The lichen flora in a region reflects the average, cumulative effects of air pollution over time; therefore, a change in the diversity could indicate a change the quality of the atmosphere. Many studies have been done to determine the effects of increasing or decreasing SO_2 on lichen abundance and diversity.

Gilbert (1968) examined lichens and bryophytes along a belt transect from the city centre of Newcastle upon Tyne to 32 km in the direction of the prevailing winds. Sulphur dioxide measurements from several standard volumetric gauges on or near the transect indicated there was a steep gradient from 200 to 40 $\mu g\ m^{-3}$ (annual average) from the city centre to 19 km out. *Evernia prunastri*, a sensitive epiphytic lichen first appeared about 13 km from the city and exhibited an increase in luxuriance, biomass and percentage cover as the distance increased up to about 27 km. Over 90% of the bryophytes and over 95% of the lichens studied exhibited a similar pattern. In contrast, the resistant epiphytic lichen, *Lecanora conizaeoides*, reached it's maximum cover on ash trees at the point where sensitive lichens were eliminated and maintained this cover into the suburbs then declined toward the city centre where it only survived in slightly sheltered sites. It was also found that sheltered environments (e.g. sheltered valleys, dense woodland) or high pH of the substrate could alleviate the toxic effects of SO_2. In addition, total sulphur in tissues of bryophytes and lichens declined as distance from the city centre increased. It was concluded that assemblages of, or individual bryophytes or lichens could be used as indicators of SO_2 pollution as long as the habitats surveyed were standardized to ensure that the environmental conditions of the habitat were not alleviating the effects of SO_2 (Table 1).

The results of a survey of lichen diversity and abundance in the Montreal area exhibited the same pattern as Gilbert's 1968 survey (LeBlanc and De Sloover, 1970). The number of species, frequency in each zone and coverage of species present generally increased as the distance from the most industrialized zones increased. The data was used to generate a single number for each site, the index of atmospheric purity (IAP), as the authors believed that the epiphytic vegetation at each site was directly related to the quality of the ambient air. However, measurements of ambient sulphur dioxide were not performed so the reduction in number of species, frequency of appearance at the different sites, and coverage or abundance at each site could not be related to SO_2 concentrations.

In a similar study in the Sudbury area, LeBlanc *et al.* (1972) surveyed 31 sites for epiphytic bryophytes and lichens and then calculated the IAP. The greater the IAP the more sensitive the epiphytes were to atmospheric pollution. The IAP's were used to separate the studied area into five zones then compared to a map that delineated 12 years of SO_2 monitoring data (12-year average concentration) into five pollution zones. It was found that the two maps were comparable; as the levels of pollution increased the IAP decreased (i.e. the frequency and diversity of epiphytes decreased). For example, zone one had an average SO_2 concentration of 79 $\mu g\ m^{-3}$ with only four species of epiphytic lichens present; whereas, in unpolluted zone five there were 40 species of epiphytic lichens (Table 1).

Another study of lichen abundance and species diversity was conducted around two gas plants that had been operating for 3 years in the Rocky Mountain House area of Alberta. The results indicated that the lichen vegetation within 1.5 to 3 km of the gas plants was already severely damaged by SO_2 emissions (Skorepa and Vitt, 1976); however, atmospheric SO_2 concentrations were not documented.

Rose and Hawksworth (1981) surveyed the abundance of lichens at 29 sites north and west of Greater London to determine if lichen populations had changed with the 50% reduction in atmospheric SO_2 since 1960. Comparison of the estimated time of recolonization with mean

annual SO_2 concentrations indicated that there was a direct response of recolonization with falling SO_2 levels. Mean annual SO_2 concentrations in Inner London declined from 200-250 $\mu g\ m^{-3}$ to less than 130 $\mu g\ m^{-3}$ between 1960 and 1980. The authors also indicated that growth rates of the species studied indicated that the recolonization occurred within the previous 3 to 7 years.

In a subsequent study, Hawksworth and McManus (1989) surveyed the same 29 sites plus 22 additional sites to determine how the lichen communities had responded to further reductions in mean winter SO_2 levels. Sulphur dioxide levels had decreased from a winter average of about 130 $\mu g\ m^{-3}$ in 1980 to 29-55 $\mu g\ m^{-3}$ for 1985 to 1987. This survey indicated that many species had recolonized throughout the study area. Of the species observed, 25 had not been recorded within 16 km of the centre of London in the twentieth century and of these, eight had not been observed for the last 200 years.

2.7.2 Physiological Response of Lichens

Kuziel (1974) used lichen and maize (*Zea mays* L.) to study the effects of SO_2 on chlorophyll content and catalase activity. Several species of epiphytic lichens were collected in the field, brought into the lab, then exposed alongside seedlings of maize (*Zea mays* L.) to 10 $mg\ m^{-3}$ SO_2 for six days (in fumigation chambers). Both were then measured for chlorophyll content and catalase activity. It was found that exposed lichens had up to 50% (*Parmelia furfuracea*) less chlorophyll than controls and up to 85% (*Ramilina fraxinea*) lower catalase activity than controls. The smallest differences were observed in maize; 6% reduction in chlorophyll and 9% reduction in catalase activity (Table 1).

Holopainen and Karenlampi (1984) studied visible injury and ultrastructural changes that occurred when the lichens *Bryoria capillaris* and *Hypogymnia physodes* L. were exposed to a range of SO_2 concentrations (140-2,860 $\mu g\ m^{-3}$). The lichens were exposed in fumigation chambers for 5.5 to 8.0 $h\ d^{-1}$ for 7 to 21 days. Visible injury, bleaching of the thalli of *H. physodes* was apparent after 4 days (22 h, fumigation at 2,860 $\mu g\ m^{-3}$) with bleaching increasing as the length of exposure increased. In contrast, electron micrographs indicated the presence of ultrastructural injury to the algal cells at all SO_2 levels, with the incidence of injury increasing as the concentration and duration of exposure increased. The type of injury observed was separated into five stages, which have been briefly described in Table 1, and related to exposure concentration and duration. While the pattern of injury for both lichens was similar, *B. capillaris* tended to have greater injury especially at the lower concentrations of SO_2. It was indicated that the ultrastructural changes observed in the lowest concentrations and shortest exposures were similar to changes observed in field material from industrial environments (Table 1).

Huebert *et al.* (1985) used *Evernia mesomorpha* Nyl. to determine the effects of low concentrations of SO_2 on net photosynthesis and respiration. The lichens were exposed to 0 to 856 $\mu g\ m^{-3}$ SO_2, for 1 to 6 h in a flow-through cuvette and net photosynthesis and respiration were measured immediately after exposure. Net photosynthesis decreased significantly when exposed to 205 $\mu g\ m^{-3}$ SO_2 for 1 h and these reductions were greater as SO_2 concentration

increased. Respiration decreased significantly after being exposed to 639 $\mu g\ m^{-3}$ SO_2 for 4 h or to 856 $\mu g\ m^{-3}$ SO_2 for 2 hours. In a second experiment, recovery of net photosynthesis was measured after a 1 or 4 h SO_2 exposure followed by a recovery period of 4 or 24 h in clean air. Exposure to 639 or 856 $\mu g\ m^{-3}$ SO_2 for 1 h required a 4 or 24 hour recovery period, respectively, for net photosynthesis to recover to near the pre-fumigation rate. When exposed for 4 h, net photosynthesis in only the 205 $\mu g\ m^{-3}$ SO_2 treatment recovered to the pre-fumigation rate. From their results the authors concluded that the most important factor in lichen response to SO_2 was exposure to short-term, high concentrations (Table 1).

Lichens in natural habitats can be exposed to dry deposition of pollutants such as SO_2 during periods of desiccation. In order to determine what effects dry deposition could have, Coxson (1988) studied the effects of rehydration on net photosynthesis and dark respiration of *Cladina mitis* (Sandst.) Hale & Culb. after a 2 or 3 week exposure of desiccated thalli to 524 or 2,619 $\mu g\ m^{-3}$ SO_2. After exposure, the dessicated thalli were rehydrated and net photosynthesis and respiration were measured for up to 800 minutes after rewetting. The pattern of photosynthetic recovery for the lichens exposed to 2,619 $\mu g\ m^{-3}$ SO_2 was no different than that of the controls for either exposure period. In contrast, the rate of recovery and maximum net photosynthesis was significantly higher in the lichens exposed to the 524 $\mu g\ m^{-3}$ SO_2 treatment (both exposure times) than in controls. Dark respiration increased significantly only in the 524 $\mu g\ m^{-3}$ SO_2 treatment (for both exposure times) after 200 minutes rehydration. It was concluded that the physiological response to periods of pollution exposure might not occur at the same time as the exposure (Table 1).

Table 1 Summary of effects of sulphur dioxide on vegetation

Note: When percent increases or decreases are given, these values are comparisons with controls unless indicated otherwise.

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Effects on Growth						
Ashenden and Mansfield (1977)	*Lolium perenne* L. (perennial ryegrass)	288	4 wk	Wind tunnel fumigation		**Wind speed 10 m min^{-1}:**
					# of fully expanded green leaves	Increased significantly greater than (>) 17%
					Dry weights	No significant differences
						Wind speed 25 m min^{-1}:
					# of fully expanded green leaves	No significant change from control
					Leaf area	Decreased significantly > 13%
					Root/shoot ratio	Decreased significantly > 34%
					Green leaf dry weight	Decreased significantly > 18%
					Dead leaves + stubble dry weight	Decreased significantly >22%
					Total shoot dry weight	Decreased significantly > 17%
					Root dry weight	Decreased significantly > 45%
					Visible injury	Some necrotic leaf lesions (1-2% of the leaf area per plant)
Constantinidou *et al.* (1976)	*Pinus resinosa* Alt (red pine seedlings)	1,310 to 10,476	15 to 120 min	Plexiglas cubes	Cotyledon chlorophyll	Reduced significantly at 1,310 $\mu g\ m^{-3}$, 120 min and at $\geq$ 2,619 for $\geq$ 30 min
					1° needle chlorophyll	Reduced significantly at $\geq$ 7,857 for $\geq$ 60 min
					1° needle dry weight	Reduced significantly at 1,310 & 2,619 $\mu g\ m^{-3}$ for 120 min and at 7,857 for $\geq$ 30 min and 10,476 at all exposure times
					# of 1° needles	Emergence appeared to be inhibited
					Cotyledon dry weight	Reduced significantly at $\geq$ 7,857 & 10,476 for $\geq$ 60 min

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Cowling and Lockyer (1976)	*Lolium perenne* L. (perennial ryegrass)	50 (daily avg)	87 d	Fumigation chambers		**% increase over no SO_2, no added soil SO_4^{2-}:**
					Shoot dry weight	66%
					Stubble dry weight	9%
					Root dry weight	31%
					Shoot sulphur	43%
						% increase over no SO_2, added soil SO_4^{2-}:
					Shoot dry weight	4%
					Stubble dry weight	8%
					Root dry weight	17%
					Shoot sulphur	24%
Cowling and Lockyer (1978)	*Lolium perenne* L. (perennial ryegrass)	55 (daily avg)	85 d	Fumigation chambers		**Low N:**
					Shoot and root dry weight, # of tillers	No change
						High N no soil S:
					Shoot dry weight	> 120% increase
					Root dry weight	> 80% increase
					# of tillers	> 20% increase
Roberts, Bruce R. (1975)	*Betula papyrifera* Marsh. (White birch)	annual avgs <10 or 70.8	3 mon – with biweekly harvests	Potted seedlings in the field	Height - % increase over 3 mon	93% - high SO_2 45% - low SO_2
					Dry weight - % increase over 3 mon	No significant difference until mid-August harvest 448% - high SO_2 229% - low SO_2
	Quercus palustris Muenchh (Pin oak)				Height - % increase over 3 mon	42% - high SO_2 73% - low SO_2
					Dry weight - % increase over 3 mon	111% - high SO_2. 261% - low SO_2

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
SO_2 Uptake and Plant S Content						
Thomas *et al.* (1944)	*Medicago sativa* L. (alfalfa)	492	628 h	Plot fumigation	Leaf extract buffering capacity	Reduced up to 46% of controls
		762	338 h		Leaf extract pH	No change
	Beta vulgaris (sugar beet) and *Medicago sativa* L. (alfalfa)	487	5.2 h d^{-1}, 60 d		Labile organic sulphur in fumigated leaves	Increased when nutrient supply deficient in sulphate No change when nutrient supply sufficient in sulphate
Visible Injury						
Katz and McCallum (1939)	11 conifer species	576	1,656 h (69 d)	Plot fumigation	Visible injury	None
		1,205	736 h (Sept 30 – Nov 5)			**First appearance of visible injury:** 530 h – Engelmann spruce
		1,833	483 h (Oct 9 – 30)			48 h – Douglas fir 120 h – White pine 162 h – Western red cedar 354 h – Engelmann spruce
		2,488	287 h (Sept 26 – Oct 8)			28 h – Douglas fir 144 h – lodgepole pine, silver fir, cedar 168 h - Engelmann spruce
		2,409	214 h (Nov 23 – Dec 2)			96 h - Douglas fir 144 h – Yellow pine, lodgepole pine
Taylor *et al.* (1986)	*Geranium carolinianum* L. (geranium)	2,095	5 h	Fumigation chamber	Leaf necrosis	Resistant ecotype – 4.6% of leaf area Sensitive ecotype – 31.1 % of leaf area
		786 to 1,571	5 h		Photosynthesis	Resistant– inhibited avg 11.5% (786 $\mu g\ m^{-3}$) and 29.9% (1,571 $\mu g\ m^{-3}$), 3-5 h exposure Sensitive – inhibited 30% at 1,571 $\mu g\ m^{-3}$
		1,179	6 h d^{-1}, 4 d wk^{-1}, 4 wk		Photosynthesis – change from controls	Resistant – increased 28% from control Sensitive – decreased 26% from control
					Photoassimilate retention in foliage	Resistant – decreased 6% from control Sensitive – increased 7% from control
		0 to 1,179			Shoot and root DW	Sensitive 10 to 25% lower than resistant ecotype

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Other Metabolic Processes						
Harvey and Legge (1979)	*Pinus contorta x banksiana* (lodgepole – jack pine)	200 to 800	0.5 h	Siemens cuvette	ATP level	No effect on chamber grown seedlings Decreased in branches from field site
		Ambient to 542		Field fumigation	ATP level	Decreased as SO_2 level increased
Horsman and Wellburn (1977)	*Rumex obstusifolius* (Broad leaved dock)	524	11 d	Wind tunnel fumigation	**Low-ambient area:**	**Activity in leaves (% of control)**
					RuDPC	Young – 65, old - 68
					GPT	Young – 138, old - 98
					GOT	Young – 127, old - 104
					Peroxidase	Young – 140, old - 135
					High-ambient area:	**Activity in leaves (% of control):**
					RuDPC	Young – 97, old - 109
					GPT	Young – 119, old - 104
					GOT	Young – 123, old - 107
					Peroxidase	Young – 114, old - 105
Olszyk and Tingey (1984a)	*Pisum sativum* L. cv Alsweet (pea)	1,310 – 2,619	2 h – dark	Fumigation chamber	Leaf injury	55 - 72% necrotic tissue
			2 h - light		Leaf injury	10 - 22% necrotic tissue
	Lycopersicon esculentum flacca Mill (tomato)	1,571 – 2,095	2 h – dark		Leaf injury	20 - 50% necrotic tissue
			2 h - light		Leaf injury	5 - 15% necrotic tissue
Olszyk and Tingey (1984b)	*Pisum sativum* L. cv Alsweet (pea)	1,833 – 2,619	2 h - light	Fumigation chamber	Leaf injury	2 to 8 times higher in FC treated plants than untreated plants
	Lycopersicon esculentum flacca Mill (tomato)	1,571	2 h - light		Leaf injury	7 times higher in FC treated plants than untreated plants
Shimazaki *et al.* (1980)	*Spinacia oleracea* L. cv. New Asia (spinach)	5,238	8 h – light	Growth cabinet fumigation	Visible injury	Appeared 2-3 h after start of fumigation
					Chlorophyll *a*	Decreased from 2 h after start of fumigation

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Chlorophyll *b*	No decrease
			10 h - dark		Visible injury	None
					Chlorophyll *a*	No decrease
Tanaka and Sugahara (1980)	*Populus euramericana* (poplar)	262	20 d	Fumigation chamber	SOD activity	Increased up to 4.4 times in leaves that were youngest at the start of fumigation
		5,238	22h		SOD activity	5 times more in young leaves than old leaves
	Spinacia oleracea L. cv. New Asia (spinach)	1,310	22h		SOD activity after treatment with a SOD inhibitor (DDTC)	65% inhibition after 2 h, reduced to 77% inhibition at end of fumigation
Effects on Pollen						
Amer *et al.* (1989)	*Vicia faba* (Faba bean)	13,095 to 654,750	5 h	Fumigation chamber	Abnormal PMC's	Significant increase (3.5 to over 50% as concentration increased)
					Nonviable pollen	Significant increase as concentration increased
		13,095	5h d^{-1} for 1, 2 or 4 d		Abnormal PMC's	Significant increase (4.6 to over 30% as exposure time increased)
					Nonviable pollen	Significant increase as exposure time increased
DuBay and Murdy (1983)	*Geranium carolinianum* L. (geranium)	1,571	8 h	Fumigation chamber	**At 90% RH:** Seed set	**Changes from the control:** Significantly reduced by 34%
					Mean number of pollen germinated on stigma	Significantly reduced by 42%
					Pollen germination	Significantly reduced by 34%
					Pollen tube growth in the style	No effects
					At 70 or 80% RH	No effects on measured parameters
Karnosky and Stairs (1974)	*Populus deltoides* Bartr. (cottonwood)	0 to 3,667	4 h Wet pollen	Fumigation chamber	Pollen tube elongation	Significantly reduced $\geq$ 786 $\mu g\ m^{-3}$
					Pollen germination	Significantly reduced $\geq$ 1,964 $\mu g\ m^{-3}$
	Pinus resinosa Ait. (Red pine) *Pinus nigra* Arnold	0 to 26,190	Dry pollen		Pollen germination and tube elongation	No effects

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	(Austrian pine) *Picea pungens* Engelm. (Blue spruce)					
			Wet pollen		Pollen germination and tube elongation	Significantly reduced to less than 20% of controls $\geq$ 3,667 $\mu g\ m^{-3}$
Keller and Beda (1984)	*Pinus mugo* Turra (Mugo pine), *Pinus sylvestris* L. (Scotch pine), *Pinus nigra* Arnold (Austrian pine)	65, 195 or 589	16 or 24 h	Fumigation chamber	Pollen germination	> 25% reduction at 195 $\mu g\ m^{-3}$ at 16 h exposure Complete inhibition at 589 $\mu g\ m^{-3}$
	Abies alba Mill. (Silver fir)		24 h		Pollen germination	> 30% reduction at 195 $\mu g\ m^{-3}$ with complete inhibition at 589 $\mu g\ m^{-3}$
Linskens *et al.* (1985)	*Petunia hybrida* Vilm. (petunia)	52,380	2 h	Petri dish	Pollen germination	45% reduction, significant
			3 h		Pollen tube growth	32% reduction, significant
Ma *et al.* (1973)	*Tradescantia paludosa* Anders (Spiderwort)	131 to 262	18 to 20 h	Sealed Plexiglas chamber	Average number of chromatid aberrations	Increased by 46.4 breaks per 100 cells in summer and by 11.7 breaks per 100 cells in fall and winter
Ma and Khan (1976)	*Tradescantia paludosa* Anders (Spiderwort)	26,190 to 2,619,000	18.5 h	Sealed Plexiglas chamber	Pollen tube growth	No effect at 26,190 $\mu g\ m^{-3}$ > 80% reduction in length at $\geq$ 130,950 $\mu g\ m^{-3}$
		196 to 130,950	18.5 h		Mitotic activity of generative nucleus	Decreased linearly with increased SO_2
O'Connor *et al.* (1987)	*Pinus radiata* (Monterey pine)	262 to 26,190	24 h	Fumigation chamber	Pollen germination	Reduced more than 50% at 26,190 $\mu g\ m^{-3}$
					Pollen tube length	50% reduction at 2,619 $\mu g\ m^{-3}$
					Activity of aspartate, glutamine alanine, and γ-amino butyrate	Reduced 24, 13, 10, and 26%, respectively at 262 $\mu g\ m^{-3}$
Varshney and Varshney (1981)	*Cicer arietinum* L. (Chick pea), *Nasturtium indicum* L. (nasturtium), *Petunia alba* Juss. (petunia),	1,310 to 13,100	1 to 5 h Dry pollen	Sealed chamber	Pollen germination	No effect up to 7,860 $\mu g\ m^{-3}$, > 10% reduction at 13,100 $\mu g\ m^{-3}$

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	Tradescantia axillaries L. (Spiderwort)					
					Pollen tube growth	Approximate 35% reduction after 5 h exposure to 13,100 $\mu g\ m^{-3}$
		26.2 to 2,620	Wet pollen		Pollen germination	Almost complete inhibition at 262 $\mu g\ m^{-3}$
					Pollen tube growth	91.3 to 96.4% reduction after 1 h at 262 $\mu g\ m^{-3}$
Multiple Pollutant (Interaction) Experiments						
Heggestad *et al.* (1986)	*Lycopersicon esculentum* cv. Jet star (tomato)	13 to 1,220	5 h d^{-1}, 5 d wk^{-1}, 57 d	Open-top chambers		**Charcoal filtered (CF)**
					Visible injury	Increased as season progressed
					Plant and fruit weight	Decreased significantly
					Ripe fruit weight	Decreased significantly
					Foliar sulphur content	Increased linearly with increased SO_2
					Fruit sulphur content	No effect
		29 to 1,226 (110 O_3)				**Non-filtered (NF)**
					Visible injury	Increased significantly over CF treatment
					Plant and fruit weight	Decreased linearly with increased SO_2
					Ripe fruit weight	Decreased linearly with increased SO_2
					Foliar sulphur content	Increased linearly with increased SO_2
					Fruit sulphur content	No effect
Houston (1974)	*Pinus strobes* L. (Eastern white pine)	65 to 1,179	6 h	Fumigation chamber	Needle elongation	Sensitive clones 48% reduction compared to controls at 65 $\mu g\ m^{-3}$
					Visible injury	Sensitive clones - all SO_2 treatments Tolerant clones – ≥ 131 $\mu g\ m^{-3}$ SO_2
		65 SO_2 + 98 O_3			Needle elongation	Sensitive clones 52% reduction compared to controls
					Visible injury	Sensitive clones only
Kress *et al.* (1982)	*Pinus taeda* (Loblolly pine)	367	6 h d^{-1}, 28 d	Fumigation chamber	Foliar injury	< 1% of the needles in any treatment

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		Plus 93 O_3, 188 NO_2	singly or in combination		Foliar injury	Sensitive trees < 4% of the needles in any treatment Non-sensitive - less injury than sensitive trees - numbers not provided
					Height	>18% reduction (sensitive), >10% reduction (non-sensitive) compared to controls in O_3 + SO_2 and O_3 + SO_2 + NO_2 treatments
Reich and Amundson (1984)	*Glycine max* (soybean)	26, 144, 288	5.25 h d^{-1} for 16 d	Field exposure	Yield	Reduction of 7% $plant^{-1}$ and 12% $hectare^{-1}$ at 288 $\mu g\ m^{-3}$
					Mass per seed	Significant 4 – 7% reduction
					Visible injury	None
		Plus: 82, 122, 157 O_3				No significant interactions
Whitmore & Mansfield (1983)	*Poa pratensis* L. (Kentucky bluegrass)	177	7 months	Fumigation chamber	Plant dry weight	55% of controls
					Shoot:root ratio	Significant increase
			11 months		Shoot dry weight	Significant increase
					Flowering	> 50% reduction
		Plus 127 NO_2	7 months		Plant dry weight	26% of controls
					Shoot:root ratio	Significant increase
			11 months		Shoot dry weight	Significant increase
					Flowering	> 50% reduction
Lichens						
Coxson (1988)	Mat-forming lichen (*Cladina mitis* (Sandst.) Hale & Culb.)	524	2 or 3 wks	Temperature-controlled cuvette	Net photosynthesis	Significant increase in rate of and maximal recovery over controls
					Dark respiration	Significant increase over controls 200 min after rewetting
		2,619			Net photosynthesis	No effect compared to controls
					Dark respiration	No effect compared to controls

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Gilbert (1968)	Epiphytic lichens (e.g. *Lecanora conizaeoides*, *Evernia prunastri*)	40 to 200	Survey of natural populations	Transect from city centre out 32 km	Luxuriance, biomass and percentage cover	Increased outward from city centre
Holopainen and Karenlampi (1984)	Epiphytic lichens - (*Bryoria capillaries Hypogymnia physodes* L.)			Fumigation chamber	**Description of stages of ultrastructural injury:**	Stage 0 - normal algal cell Stage 1 - mitochondria swollen & deformed Stage 2 - swelling of chloroplasts, degeneration of nucleus, mitochondrial cristae and matrix Stage 3 – Mitochondria and nucleus badly degenerated, thylakoids appear stretched Stage 4 – All cell organelles badly injured but their outlines were still visible Stage 5 – no organelles visible, cell contents collapsed
		140	8 h d^{-1} - 21 d		Stage of injury after indicated exposure:	14-21 d – stage 1
		290	5.5 h d^{-1} - 9 d			4 d – stages 0 to 1 9 d – stages 1 to 3
		860	5.5 h d^{-1} - 6 d			3 d – stages 1 to 2 6 d – stages 2 to 3
		2,860	5.5 h d^{-1} - 7 d			1 d – stages 1 to 2 4-7 d – stages 4 to 5
					Visible injury	Bleaching of thalli visible after 4 d exposure
Huebert *et al.* (1985)	*Evernia mesomorpha* Nyl.	0 to 856	1 to 6 h	Flow-through cuvette	Net photosynthesis	Significant reduction at 205 $\mu g\ m^{-3}$ for 1 h or more – reductions increased as concentration increased
					Respiration	Significant reduction at 639 $\mu g\ m^{-3}$ or higher, for 4 h or more
		0 to 1342	1 h		Recovery of net photosynthesis	639 $\mu g\ m^{-3}$ - 4 h after exposure recovery almost complete 856 $\mu g\ m^{-3}$ - 24 h after exposure recovery almost complete Higher concentration, recovery incomplete
		0 to 856	4 h		Recovery of net	205 $\mu g\ m^{-3}$ - only treatment to recover to

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					photosynthesis	pre-treatment levels 4 h after exposure
Kuziel (1974)	Epiphytic lichens	10,000	6 days	Fumigation chamber	Chlorophyll	50% reduction *(Parmelia furfuracea)* compared to controls
					Catalase activity	85% reduction (*Ramilina fraxinea*) compared to controls
	Zea mays L. (Maize)				Chlorophyll	6% reduction compared to controls
					Catalase activity	9% reduction compared to controls
LeBlanc *et al.* (1972)	Epiphytic lichens	79	12-y avg. ambient	Natural ecosystem	Number of species	Reduced to 4 from unpolluted area
		52 to 79			Number of species	Reduced to 14 from unpolluted area
		26 to 52			Number of species	Reduced to 21 from unpolluted area
		< 13			Number of species	40 (considered an unpolluted area)

PART II

3.0 LITERATURE 1990 TO 2002

This section summarizes selected primary literature published from 1990 to 2002 on the effects of SO_2 exposure on vegetation. Table 2 summarizes the results of the studies.

3.1 Effects on Growth

Qifu and Murray (1991) used potato (*Solanum tuberosum*) to study the interactive effects of soil water stress and SO_2. Plants were exposed to 288 or 786 $\mu g\ m^{-3}$ SO_2, for 105 days in closed top field chambers for 4 h per day under well-watered or water stressed conditions. Visible symptoms appeared after 9 weeks exposure to 288 $\mu g\ m^{-3}$ and after six weeks exposure to 786 $\mu g\ m^{-3}$. At harvest, the leaf S content of well-watered plants had increased by more than 100% and 125% in the 288 and 786 $\mu g\ m^{-3}$ SO_2 treatments, respectively. When water stressed, the lower SO_2 treatment had little effect on S content; whereas, the 786 $\mu g\ m^{-3}$ treatment resulted in a 100% increase in leaf sulphur. Leaf chlorophyll of 35-day-old leaves from well-watered plants decreased significantly, by approximately 30% at 288 $\mu g\ m^{-3}$ SO_2 and by 40% at 786 $\mu g\ m^{-3}$ SO_2. In contrast, water stress resulted in a maximum chlorophyll loss of 11% in the 786 $\mu g\ m^{-3}$ SO_2 treatment. Exposure of well-watered potato plants to 786 $\mu g\ m^{-3}$ SO_2 resulted in significant decreases in leaf (25%) and tuber (35%) dry weight compared to controls. In contrast, dry weight reductions of water stressed plants did not usually occur when plants were exposed to SO_2. It was suggested that the reduced response of water stress plants when exposed to SO_2 might be a result of increased stomatal resistance in response to mild water stress that may have reduced SO_2 uptake.

Coleman *et al.* (1990) presented results on the variability of biomass production for wild radish (*Raphanus sativus* x *raphanistrum*) and cultivated radish (*Raphanus sativus*) cv. Cherry Belle when plants were exposed to SO_2. Plants were exposed in fumigation chambers during a 10 h light period to 262, 629 or 1,048 $\mu g\ m^{-3}$ SO_2 for 24, 30 or 35 days. They found that variability associated with biomass production increased as SO_2 exposure increased while biomass itself was only significantly reduced in one experiment using Cherry Belle. It was indicated that genetic differences between the individual plants (differential sensitivity to SO_2) might be the reason for the increased variability as the SO_2 concentration increased.

Top-covered chambers were used to investigate visible injury, growth, and stomatal resistance of soybean (*Glycine max* L.) cv. Buchanan when exposed to salt stress followed by SO_2 or SO_2 followed by salt stress (Qifu and Murray, 1994). Plants were exposed to 380 or 786 $\mu g\ m^{-3}$ SO_2 for 5 h per d, for 3 weeks followed by exposure to low (320 g/200 L) or high (540 g/200 L) salt stress for an additional 3 weeks. The reverse conditions were also looked at. They found that exposure to either concentration of SO_2 alone resulted in a significant increase in chlorophyll content of leaves and the shoot:root ratio, and significant decreases in leaf area, root and shoot dry weight, and fresh weight of root nodules. Subsequent exposure to salt stress resulted in reduced chlorophyll in all treatments. Exposure to high salinity after SO_2 resulted in visible injury within 5 days, death of all plants from the high SO_2 treatment within 12 days, and death of

half of the low SO_2 treatment plants by the end of 3 weeks. In contrast, when high SO_2 exposure was preceded by low salinity stress, the adverse effects of high SO_2 were mitigated for all growth variables. It was concluded that prior treatment to SO_2 physiologically weakened plants mainly through the increased shoot:root ratio (root surface inadequate to supply water needs of shoots).

Kropff (1990) performed field exposures with an open-air fumigation system and used the data in a crop growth model, which was used to interpret and explain the observed yield loss by quantifying the contribution of different physiological effects. Fumigation of broad bean (*Vicia faba* L.) with 74 $\mu g\ m^{-3}$ SO_2, for the growing season, resulted in a 9% decrease in total dry matter accumulation and an 10% decrease in pod yield at the final harvest. These losses were accompanied by visible injury (brown/red spots), which progressed from the oldest leaves upward and also resulted in some leaf abscission. When exposed to 165 $\mu g\ m^{-3}$ SO_2, dry matter was reduced by 17% and yield was reduced by 23%. The author indicated the loss of dry matter production was primarily a result of loss of green leaf area in exposed plants.

In an open-top chamber study examining sulphur accumulation, and alteration of growth and yield in barley (*Hordeum vulgare* L.) cv. Schooner, Murray and Wilson (1990) found that exposure to 110 $\mu g\ m^{-3}$ SO_2 for 4 h day^{-1} for 79 days produced an increase in mean shoot height and weight of approximately 10%. This increase in growth was attributed to a fertilizer effect of SO_2. At 317 $\mu g\ m^{-3}$ SO_2 or higher, significant linear decreases in barley growth (height, weight, and number of tillers), and yield (head number and weight) were observed. These decreases were proportional to the concentration of the SO_2 exposure. In addition, the shoot sulphur content increased linearly from 0.14% in the control plants to 0.77% at 1,354 $\mu g\ m^{-3}$ SO_2.

Colls *et al.* (1992) used and open-air fumigation system to expose winter barley (*Hordeum vulgare* L.) cv. Igri to a single dose (defined as concentration x time) of SO_2, to determine if concentration peaks or long-term averages had the greatest effects on the plants. The treatments were based upon achieving an equivalent dose of 534 $\mu g\ m^{-3}$ days. The treatments were: continuous exposure to 89 $\mu g\ m^{-3}$ for 6 days, 178 $\mu g\ m^{-3}$ for 3 days followed by ambient air for 3 days, or 534 $\mu g\ m^{-3}$ for 1 day followed by 5 days of exposure to ambient air. This six-day cycle was repeated 24 times during the growing season. There were no effects on shoot dry weight accumulation or on grain yield in any treatment. The lack of effects due to the concentration peaks was attributed to plants being able to metabolize excess sulphate during the SO_2 free days.

Open-top chambers were used to investigate the response of barrel medic (*Medicago truncatula* Gaerm.) cv. Paraggio when exposed to SO_2 (Murray and Wilson, 1991). Plants were exposed to 107 to 1,349 $\mu g\ m^{-3}$ SO_2 for 4 h day^{-1}, 7 d $week^{-1}$ for 72 days. Growth was affected very little at concentrations up to 314 $\mu g\ m^{-3}$ SO_2 (<10% reduction); however, at 668 $\mu g\ m^{-3}$ SO_2 there was a 40 to 50% reduction in growth that was accompanied by an approximately 85% increase in the S concentration. At 1,349 $\mu g\ m^{-3}$ SO_2, there was little or no growth. One important aspect of the response of barrel medic was the significant reduction in flowering as SO_2 concentration increased. It was indicated this could have implications for pasture maintenance, as self-sown seed is the method by which annual medics regenerate each year.

In a field study of long-term effects of emissions on two sites near a point source of SO_2 (sour gas plant), and a control site, Clapperton and Parkinson (1990) studied the inoculum potential of vesicular arbuscular mycorrhizae (VAM). The study sites were 5 (site 1) and 13 km (site 2) downwind of the plant while the control site (site 3) was 20 km upwind. Daily average SO_2 concentrations, May to September were 80, 27 and <27 $\mu g\ m^{-3}$. When timothy (*Phleum pratense*) seedlings were grown for 4 weeks in soil from the 3 sites, it was found that the rate of infection by VAM was significantly lower at the 2 sites closer to the gas plant. The number of spores (inoculum potential) also decreased at sites closer to the plant; 714, 872 and 1,372 per 100 g dry weight soil for sites 1, 2 and 3, respectively. It was indicated that the reduction in inoculum potential was probably a result of lowered productivity of the host (plants at the study sites) and reduced host productivity was probably due to long-term exposure to SO_2.

Ashenden *et al.* (1996) conducted greenhouse studies of SO_2 effects on growth of 41 British herbaceous species to determine if the species differed in their sensitivity to SO_2. Plants were exposed to a constant background concentration of 262 $\mu g\ m^{-3}$ SO_2 with peaks applied during daylight. During the first 4 weeks, peaks were 524 $\mu g\ m^{-3}$ for 2 h, twice per week. For the next 3 weeks 786 $\mu g\ m^{-3}$ was applied for 3 h, 3 times per week. Finally, for the last 3 weeks peaks of 786 $\mu g\ m^{-3}$ were applied for 3 h, 5 times per week. These concentrations were chosen to maximize any growth differences between the tested species. Of the 18 statistically significant responses in terms of total dry mass reductions, there was an average decrease of 43%. The mean response of all 41 species was a 25% decrease in total dry mass. Of the seven statistically significant responses of total leaf area, there was an average decrease of 40%. The mean response for all 41 species was a decrease of 10% in total leaf area. For leaf area ratio (total leaf area:total dry mass), there was an average increase of 45% for the 20 statistically significant responses, and an average increase of 23% for all 41 species. An average decrease of 36% in the root:shoot ratio occurred due to SO_2 exposure (for the 13 species with statistically significant responses); whereas, for all 41 species the decrease was 14%. The authors indicated that while there were differences in growth response of the species tested here, the same responses may not be observed under field conditions because SO_2 concentrations in the field are not expected to be as high as those used in this study. Plants growing in natural communities may also respond differently than plants grown in individual containers. In addition, the nutrient supply in this study was non-limiting while in the field nutrients may be limiting and may alter responses to SO_2.

Julkunen-Tiitto *et al.* (1995) studied the effects of SO_2 exposure on growth and secondary metabolism (production of phenolics and soluble sugars) in six clones of willow (*Salix mysrsinifolia*). A disruption in secondary metabolism could cause a reduction in plant defence mechanisms to herbivory and microorganisms. Clones were exposed, in fumigation chambers, to 300 $\mu g\ m^{-3}$ SO_2 for 7 h day^{-1}, 5 days $week^{-1}$, for 3 weeks. Salicin and chlorogenic acid content were significantly decreased by 15% to over 70% (depending on clone) while there was no significant effect on salicortin, 2'-O-acetylsalicortin, (+)-catechin and two unknown phenolics. Since SO_2 exposure did not affect salicortin and 2'-O-acetylsalicortin (key molecules in the defence chemistry it was concluded that willow resistance to herbivory and microorganism attack was not reduced. Glucose, fructose and sucrose contents were not significantly affected. They found that willow exposed to 300 $\mu g\ m^{-3}$ SO_2 produced a 14% to 48% greater phytomass

(leaf, stem, and root dry weights) compared to control plants. This increased growth was attributed to SO_2 acting as an additional nutrient source.

Clarke and Murray (1990) studied the effects of long-term SO_2 exposure on growth and development of *Eucalyptus rudis* Endl. The plants were exposed to 132 and 274 $\mu g\ m^{-3}$ SO_2 for 8 h day^{-1} in open-top chambers, for 17 weeks. There was no effect on S content at the lower concentration of SO_2, but the 274 $\mu g\ m^{-3}$ treatment significantly increased total leaf S. They found that a 17-week exposure to 132 $\mu g\ m^{-3}$ SO_2 increased the height, average leaf area, and average dry weight of leaves. The increased leaf area and dry weight were attributed to increased size of leaves, as there was no effect on total number of leaves. Sulphur dioxide levels of 274 $\mu g\ m^{-3}$ had no effect on plant height, average leaf area and average dry weight of leaves, but produced an increased rate of leaf abscission. However, new leaf production replaced the fallen leaves and the total number of leaves did not change.

Agrawal and Verma (1997) investigated whether varying the levels of nitrogen (N), potassium (K), and phosphorous (P) in the growth medium would affect the response of wheat (*Triticum aestivum* L.) cvs. Malviya 206 and Malviya 234 to SO_2. Wheat was grown in the field with 6 nutrient treatments applied after a basal nutrient addition. Treatments were: no additional nutrients, a recommended application rate, and 4 combinations of N, P, and K. Thirty days after sowing plants were exposed, in open-top chambers to $390 \pm 20\ \mu g\ m^{-3}$ SO_2 for 4 h day^{-1}, 5 d $week^{-1}$, for 8 weeks. Visible injury symptoms appeared earlier and were the greatest in both cultivars grown with no additional nutrients. Unfertilized plants exposed to SO_2 had the greatest dry weight, height, and yield reductions while plants grown with recommended or 2 times the recommended levels of NPK were able to alleviate SO_2 effects to the greatest extent. Leaf area and total chlorophyll content decreased significantly when plants were exposed to SO_2. Ascorbic acid was significantly reduced in treated plants, which was attributed to consumption of ascorbic acid for removal of free radicals generated when SO_2 entered foliar tissue. Sulphur dioxide treatment also resulted in an increase in sugars that was associated with a decrease in starch, which suggested increased hydrolysis of polysaccharides for use in the repair process and synthesis of other metabolites. Sulphate-S increased significantly in treated plants with the greatest increase observed in plants grown with no additional nutrients. An increase in the root:shoot ratio was also observed in SO_2 treated plants that suggested a modification in the carbon allocation pattern when plants were exposed to SO_2. They concluded that both nutrient deficiency and SO_2 caused the observed reductions of measured parameters but that the addition of NPK in different combinations was able to ameliorate the adverse effects of SO_2.

3.2 Sulphur Dioxide Uptake and Plant Sulphur Content

In some species foliar sulphur and sulphate content may be used as an indicator of SO_2 exposure. Agrawal and Singh (2000) studied six species of tropical trees from a low rainfall area along a pollution gradient (seasonal average of 49 to 233 $\mu g\ m^{-3}$ SO_2) around two coal-fired power plants in India. The focus of this study was to determine the effects of the power plant emissions on the nutrient status of trees transplanted from a plantation into an area in which most of the natural forest had been destroyed. Two species of evergreens (mango, *Mangifera indica*; eucalyptus,

Eucalyptus hybrid) and four species of deciduous trees (guava, *Psidium guajava*; cassod tree,*Cassia siamea*; flame-tree, *Delonix regia*; bougainvillea, *Bougainvellea spectabilis*) were studied. Total foliar sulphur content was higher in all six species at the most exposed location compared to the reference location. In deciduous species there was a greater increase in the foliar sulphate-S content after the onset of new leaves during the summer, which the authors indicated was possibly due to translocation of sulphur from woody plant parts.

In an attempt to assess the critical level of SO_2 in the air which would cause adverse effects on vegetation, Manninen and Huttunen (1995) examined total sulphur content in needles of Scots pine (*Pinus sylvestris* L.) growing at ambient SO_2 levels. Monthly mean concentrations ranged from 10 to 100 $\mu g\ m^{-3}$ in 1980, 8 to 61 $\mu g\ m^{-3}$ in 1985 and 0 to 95 $\mu g\ m^{-3}$ in 1989. They found significant correlations between total sulphur content of the two youngest age-classes of needles (7 and 19 months) and the monthly mean and daily mean ambient SO_2 concentrations. There also appeared to be a relationship between total needle sulphur content and the extent of cuticular damage on current year growth and 12-month-old needles (i.e. decreased damage with decreased sulphur content). Sample size was small here so no statistical analysis was performed.

The Liphook Forest Fumigation Project was established to determine the long-term effects of exposing specific tree species to realistic levels of SO_2 and O_3 within field fumigation plots. One of the studies investigated sulphur and nutrient accumulation in needles of Sitka spruce (*Picea sitchensis* Bong. Carr.), Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.) exposed to 34 or 58 $\mu g\ m^{-3}$ SO_2 for 43 months (Shaw and McLeod, 1995). Plants were also exposed to 1.3 times the ambient ozone concentration from spring to December of each year. Ozone concentrations were not given but the authors indicated there was little evidence for effects of ozone on the foliar chemistry. In all three species, both SO_2 concentrations resulted in increased sulphur content and ratio of sulphur to cation levels, in exposed needles.

3.3 Visible Injury

Clapperton and Reid (1994) collected genotypes of timothy (*Phleum pratense*) from two field sites, located in the foothills of southern Alberta, 5 and 20 km from a gas plant. Plants were screened for SO_2 sensitivity in experiments conducted in closed fumigation chambers. In the first experiment, plants were exposed to 393 to 524 $\mu g\ m^{-3}$ SO_2. The experiment was terminated after 3 weeks when sensitive plants developed chlorotic areas, browning of the leaves and dead tissue. In a second experiment, plants were exposed to 170 $\mu g\ m^{-3}$ SO_2 and the experiment was terminated when plants showed the first signs of damage (two weeks). Plants were considered tolerant when they exhibited no signs of visible injury and no significant decreases in shoot or root dry weights compared to control plants. Subsequent field trials that were also done in this study are not described here, because ambient SO_2 concentrations were not provided.

3.4 Photosynthesis

Several studies have been conducted on the effects of SO_2 on metabolic processes in plants that can affect photosynthesis or be altered as a result of changes in photosynthesis. The following studies examined one or more of the processes closely related to photosynthesis, including: stomatal conductance, photochemical efficiency, carbon dioxide assimilation, chlorophyll content, dark respiration, and carbohydrate metabolism.

The effects of SO_2 on photosynthetic carbon metabolism in winter barley (*Hordeum vulgare* L.) cv. Igri were investigated by assaying the activity of key enzymes in carbon fixation and sucrose synthesis in a 2-year field experiment at Littlehampton, Great Britain (Montiel-Canobra *et al.*, 1991). The crop was exposed to three SO_2 concentrations plus an ambient control using an open air fumigation system. In the first year of the study samples were collected from the ambient (18 $\mu g\ m^{-3}$) and high (126 $\mu g\ m^{-3}$) SO_2 treatments and in the second year samples were collected from the ambient, medium (73 $\mu g\ m^{-3}$) and high (100 $\mu g\ m^{-3}$) SO_2 treatments. Significant reductions in flag leaf area of 20% (for each year) and flag leaf dry weight of 23 and 21% were observed in the 1st and 2nd seasons, respectively. The data also indicated there was a delay in flag leaf emergence. The authors indicated that the reduction in size and weight of flag leaves could restrict grain filling due to a reduction in photosynthate production of the leaves. Exposure to the highest SO_2 treatment in each growing season (100 and 126 $\mu g\ m^{-3}$), resulted in a significant reduction in fructose-1,6-bisphosphatase (FBPase) activity being observed for the post-anthesis period. It was concluded that reductions in FBPase could influence photosynthetic carbon partitioning in leaves, assimilate distribution, and export of fixed carbon to developing tissues. There were no significant treatment effects on chlorophyll content, phosphoribulokinase (PRK), NADP-dependent glyceraldehyde-phosphate dehydrogenase (NADP-GPD) or phosphoglycerate kinase (PGK) in either growing season.

Lorenzini *et al.* (1995) evaluated the gas-exchange response of two-year-old seedlings of oak (*Quercus pubescens* Wild.) and Turkey oak (*Quercus cerris* L.) exposed to 73, 160 and 244 $\mu g\ m^{-3}$ SO_2 for 23 weeks, in fumigation chambers. After 11 weeks of exposure *Q. pubescens* exhibited a significant linear decrease in photosynthetic activity, stomatal conductance, transpiration rate, and water use efficiency. In addition, the vapour pressure deficit increased with increasing SO_2 concentration, and the internal/ambient CO_2 concentration ratio was not affected. For *Q. cerris* there was a significant linear decrease in photosynthetic activity, vapour pressure deficit, and water use efficiency, but there was no effect on stomatal conductance or the transpiration rate. The internal/ambient CO_2 concentration ratio increased 15% at 244 $\mu g\ m^{-3}$ SO_2. At the conclusion of the 23 week exposure; tree height and leaf area were not affected in either species; whereas, foliar starch and total S content increased linearly with increasing SO_2 concentration for both species (up to four times more than controls with no visible injury symptoms). Leaf dry weight for *Q. cerris* was linearly depressed as SO_2 increased. The authors attributed the differences in response of the two *Quercus* species to intrageneric variability of physiological response to SO_2. It was also suggested that this type of exposure should be conducted with mature trees to determine if seedling and mature tree responses differed.

Veeranjaneyulu *et al.* (1991) used photoacoustic techniques (detection of heat and photosynthetic O_2 pulses from leaves) to study responses of photosynthetic activity (oxygen evolution and energy used in photosynthesis (PES)) to 131 to 5,238 $\mu g\ m^{-3}$ SO_2 in the broad-leaf tree, sugar maple (*Acer saccharum*). They found that at 131 $\mu g\ m^{-3}$ SO_2, O_2-evolution and PES increased by 65% and 24%, respectively. However, at 5,238 $\mu g\ m^{-3}$ SO_2, these two parameters decreased by 50% and 22%, respectively compared to controls. It was concluded that the enhanced O_2 evolution and PES observed at the lowest concentration was a result of detoxification of SO_2, which in turn stimulated photosynthetic electron transport and that the higher SO_2 concentrations inhibited this electron transport reduced O_2 evolution and PES.

In order to determine if airborne pollutants such as SO_2, copper (Cu), and nickel (Ni) affected the photosynthetic efficiency of natural forests, Odasz-Albrigtsen *et al.* (2000) studied 16 sites along a pollutant gradient. Ambient SO_2 measurements for 1989 to 1992 were modelled by the Norwegian Institute of Air Research to cover the entire region (100x100 m grid). Means ranged from 30 $\mu g\ m^{-3}$ (7 km from smelters) to non detectable at the control site 386 km away. Concentrations of Ni and Cu, measured at ground level were highest 7 km from the smelters, 8.0 and 5.0 $mg\ m^{-2}$, respectively. Both metals were non detectable at the control site. Chlorophyll fluorescence was measured in the field and photosynthetic efficiency was estimated using these measurements. It was found that photosynthetic efficiency was generally lower closer to the smelters; although, most of the affected plants failed to show any visible signs of stress or damage. While photosynthetic efficiency was reduced in more than 50% of species investigated, only six species exhibited a weak but significant correlation that indicated photosynthetic efficiency decreased with an increase in SO_2 levels. The six species were: birch (*Betula pubescens*), bilberry (*Vaccinium myrtillus*), crowberry (*Empetrum hermaphroditum* Hagerup), Branch lichen (*Hypogymnia physodes* L.), ground lichen (*Cladina* spp.) and snow-level lichen (*Parmelia olivacea*). Pine (*Pinus sylvestris* L.) exhibited no decrease in photosynthetic efficiency, which was attributed to mitigation of pollution by upper branches (measurements were taken in the lower canopy). Photosynthetic efficiency of all lichen species tested in the mixed forest was inversely correlated with Ni and Cu deposition. Although the vegetation was exposed to SO_2 and metals (Cu, Ni) simultaneously, the potential for interactions was not discussed.

To determine the effect of SO_2 on chlorophyll content, Panigrahi *et al.* (1992) exposed rice (*Oryza sativa* L.) at 20, 40, 60, 80 and 100 days old; and mung bean (*Phaseolus aureus* R.) cv. Dhauli at 15, 30, 45 and 50 days old to 655 to 5,240 $\mu g\ m^{-3}$ SO_2 for 6 to 48 h. For both species, chlorophyll content decreased significantly with increased SO_2. In addition, chlorophyll content decreased in each SO_2 treatment as the exposure time increased. It was found that chlorophyll content decreased between 20 and 40% in rice and mung bean at SO_2 concentrations of 655 and 1,310 $\mu g\ m^{-3}$ respectively. Exposure to the highest concentration of SO_2 (5,240 $\mu g\ m^{-3}$) resulted in almost complete destruction of chlorophyll. It was concluded that a decrease in chlorophyll leads to a decrease in growth parameters including biomass, productivity, and yield; however, growth parameters were not measured in this study so these could not be directly related to the observed chlorophyll reductions.

In a study designed to examine changes in leaf gas exchange resulting from SO_2 exposure, Gerini *et al.* (1990) exposed maize (*Zea mays* L.), in fumigation chambers to 113, 186 or 291 $\mu g\ m^{-3}$ SO_2 for 4 weeks. A 20% decrease in photosynthetic activity was observed in plants exposed to 113 and 186 $\mu g\ m^{-3}$ SO_2. At 291 $\mu g\ m^{-3}$ SO_2, photosynthetic activity was decreased by 10% compared to control plants. Stomatal conductance, transpiration rate, and intercellular/ambient CO_2 were enhanced at the lowest SO_2 treatment, but then declined to near control levels at the highest SO_2 treatment. In contrast, water use efficiency and CO_2 assimilation rate declined at the lowest concentration then increased (but not back to control levels). Sulphur dioxide levels used in the study were representative of ambient SO_2 levels observed in the environment. The decrease in photosynthetic activity was attributed to reduced mesophyll assimilation capacity. Stomatal effects were ruled out as stomatal conductance and intercellular CO_2 were enhanced at these levels of SO_2.

Darrall (1991) utilized an open-air fumigation system to examine the effects of SO_2 (ambient, low, medium, and high) on photosynthesis, dark respiration, transpiration, stomatal conductance, and internal CO_2 concentration and discussed observed changes to effects on grain yield in winter barley (*Hordeum vulgare* L.) cv Igri. Experiments were conducted for 3 years and the SO_2 concentrations varied within each year. The average concentrations for the highest SO_2 treatment, for each year were 100, 113, and 126 $\mu g\ m^{-3}$. Although SO_2 significantly increased net photosynthesis on some occasions, significant decreases were observed on other occasions. Most of the photosynthetic changes were transient and were attributed to simultaneous changes in stomatal conductance and transpiration. Dark respiration was significantly enhanced at 84 and 113 $\mu g\ m^{-3}$ SO_2 (medium and high SO_2 plots, respectively). It was suggested that increases in dark respiration could have resulted from enhanced detoxification and repair processes. No significant effect on grain yield or total plant weight occurred as a result of SO_2 treatment. The author also noted that powdery mildew infested the lower leaves of the plants, which may have affected the results of the experiment.

Ranieri *et al.* (1999) investigated long-term exposure of barley (*Hordeum vulgare* L.) cvs. Panda and Express to 210 $\mu g\ m^{-3}$ SO_2, in a greenhouse, to establish if negative impacts of SO_2 could be linked to specific changes in the photosynthetic apparatus. Exposure for 75 days did not result in visible injury to either cultivar, Panda or Express. Results are given as per cent compared to controls. However, the 75 day exposure to 210 $\mu g\ m^{-3}$ SO_2 decreased photosynthetic activity by 29 and 49% in cultivars Panda and Express, respectively. Stomatal conductance was reduced by 56% (Panda) and 58% (Express), and whole electron transport chain activity was reduced by 27% (Panda) and 29% (Express). Electron transport activities of photosystem I and II were reduced by 7 and 11%, respectively, in Panda and 18 and 24% respectively, in Express. Significant decreases in leaf pigments were also observed. For example, chlorophyll *a* decreased by 44% (Panda) and 10% (Express), while carotenoids decreased by 46% (Panda) and 10% (Express). Pigment-protein complexes from thylakoid membranes did not show any qualitative or quantitative differences between control and SO_2 exposed plants. The authors concluded that the reduction in photosynthetic activity could be attributed to stomatal closure and a generalized negative impact on the photosynthetic apparatus.

3.5 Other Metabolic Processes

Gupta *et al.* (1991) studied the effects of SO_2 exposure on soybean (*Glycine max*) cv. Elf to determine effects on abscisic acid (ABA) content at the end of the exposure period and after a recovery period of 18 hours. Thirty day old soybean seedlings were exposed in growth chambers to 131, 524 or 1,048 $\mu g\ m^{-3}$ SO_2 for 1, 2 or 4 hours. No visible injury was observed at a SO_2 level of 131 $\mu g\ m^{-3}$. Although the authors indicated there was slight injury (chlorosis) of top leaves after the 18 hr recovery period in the 524 $\mu g\ m^{-3}$ SO_2 treatment but it was not indicated which exposure length resulted in the observed symptoms. Damage (leaf curl and necrotic areas) was also visible in the 1,048 $\mu g\ m^{-3}$ SO_2 treatment within 4 h of start of treatment. They found that both exposure concentration and duration significantly increased abscisic acid (ABA) content in leaves. At a SO_2 concentration of 131 $\mu g\ m^{-3}$, ABA content increased 28% after 1 h, 87% after 2 h and 141% after 4 h exposure. The 18 hour recovery period resulted in a reduction of ABA levels in all treatments, but ABA levels were still higher than the controls.

Madamanchi and Alscher (1991) examined antioxidants and antioxidant enzymes in an attempt to understand the metabolic differences of two cultivars of pea (*Pisum sativum* L.) known to differ in their sensitivity to SO_2 exposure (cvs. Progress, insensitive and Nugget, sensitive). Plants were exposed in continuously stirred tank reactors to 2,095 $\mu g\ m^{-3}$ for 210 min after SO_2 reached the target concentration (this took about 40 min.). Total glutathione (ratio of exposed/control) content increased from 1.11 (at 0 min.) to 2.04 (at 210 min. exposure) in Progress and 1.42 (at 0 min.) to 1.69 (at 210 min. exposure) in Nugget. Reduced glutathione (GSH) increased in Progress from 1.11 to 1.93 and in Nugget from 1.37 to 1.59 for 0 and 210 minutes exposure, respectively. No significant effects were found on ascorbic acid or oxidized glutathione content. Superoxide dismutase activity increased 90% in Progress, but was unaffected in Nugget. Mean glutathione reductase activity increased 35% and 21% in Progress and Nugget, respectively. The authors suggested that the significantly increased glutathione content, glutathione reductase, and superoxide dismutase activities of Progress might be a part of its metabolic resistance to SO_2 exposure.

Rao and Dubey (1990) studied antioxidant production and its role in protecting four tropical tree species (ber, *Zizyphus mauritiana*; jamun, *Syzygium cumini*; neem, *Azadirachta indica*; mango, *Mangifera indica*) from air pollution. Four exposure sites were selected downwind from an industrial source, while the control site was 10 km upwind. Samples were collected once a month for 12 months. The monthly average SO_2 concentration at the 4 sites ranged from 48 to 90 $\mu g\ m^{-3}$. The authors considered SO_2 to be the primary pollutant with respect to the plants responses but did not rule out the possible interactive effects of the other pollutants. Sulphate accumulation in the leaves corresponded with the ambient SO_2 level. When exposed to 90 $\mu g\ m^{-3}$ SO_2, the sulphate content in the leaves increased by 72%, 69%, 65%, and 92% for ber, jamun, neem, and mango, respectively, in comparison to the control site. Increased sulphate content in the four species ranged from 26% to 48% at the site with an ambient level of SO_2 (48 $\mu g\ m^{-3}$). Stomatal conductance decreased by 26 to 28% in the four species at the site with the highest SO_2 level in comparison to the control site. Oxidation of proteins, superoxide dismutase activities and peroxidase activities increased in all four species. The magnitude of the response varied with species and was related to the ambient SO_2 concentration. It was concluded

that increased peroxidase and superoxide dismutase activities could increase SO_2 tolerance under field conditions.

Borland and Lea (1991) investigated the long-term effects of 39, 73, and 100 $\mu g\ m^{-3}$ SO_2 (growing season means) on nitrate reductase, nitrite reductase, glutamine synthetase, glutamate dehydrogenase, glutathione reductase activity and total glutathione content in winter barley (*Hordeum vulgare* L.) cv. Igri. Experiments were conducted using an open-air fumigation system at Littlehampton, Great Britain. Nitrate reductase activity in tissues harvested in February, March and April was significantly decreased by 100 $\mu g\ m^{-3}$ SO_2. Nitrite reductase activity was relatively constant except for significant increases in April (at 100 $\mu g\ m^{-3}$ SO_2) and May (at 39 $\mu g\ m^{-3}$ SO_2). There was no effect at any concentration of SO_2 on glutamine synthetase or glutathione reductase. Exposure to SO_2 significantly increased glutamate dehydrogenase activity in samples obtained in December, January and June. Total glutathione varied with the season but there was no accumulation with SO_2 exposure. It was concluded that the concentrations of SO_2 were too low to generate a significant response.

Chauhan (1990) performed a study on the early diagnosis of SO_2-stress and the mechanism of SO_2 damage in crop plants by measuring volatile emissions from treated tissues. Emissions from tomato (*Lycopersicon esculentum* Mill.), mung bean (*Vigna radiata* L.) and maize (*Zea mays* L.) were measured after exposure to 262 $\mu g\ m^{-3}$ for 2 h per day or 524 $\mu g\ m^{-3}$ for 1 h per day. Tomato and maize were exposed for 60 days and mung bean was exposed for 45 days as it has a shorter life span. Ethylene, ethane, acetaldehyde, and ethanol were measured at 15 day intervals. Ethylene emissions increased substantially in all three species, until visible injury symptoms (chlorosis followed by necrosis) appeared, after which ethylene declined until the end of the exposure. Ethane emissions were detected just prior to the appearance of visible injury symptoms and increased as injury increased. It was suggested that ethane production was a result of lipid peroxidation caused by sulphate oxidation. To verify this, an additional experiment with mung bean was performed to establish if the addition of antioxidants would reduce SO_2 induced damage. Antioxidants substantially reduced ethylene and ethane production supporting the idea that lipid peroxidation was caused by free radicals resulting from sulphite oxidation. Acetaldehyde and ethanol emissions increased as exposure duration increased up to 45 days, but emissions declined after the appearance of visible injury symptoms. As acetaldehyde and ethanol are not normal by-products of aerobic metabolism the author suggested that their production was a result of SO_2 alteration of respiratory metabolism. The rates of emissions of ethane, acetaldehyde, and ethanol were related to the degree of SO_2 resistance displayed by the species in the study (the greater the resistance the greater the rates of emissions).

Bernardi *et al.* (2001) studied levels of soluble leaf proteins and the response of the superoxide dismutase (SOD) complex of bean plants (*Phaseolus vulgaris* L.) cv. Groffy after exposure to SO_2 (79, 157 or 236 $\mu g\ m^{-3}$) for 2, 4, or 7 days. No visible injury symptoms were observed in any of the treated plants. Newly synthesized polypeptides were detected in all treatments and there were quantitative differences between the control and treated plants for 6 other protein subunits. The authors indicated that the observed changes in protein synthesis might be linked to a SO_2 resistance mechanism. In addition, SO_2 exposure induced the activation of an additional SOD isoform, which when tested exhibited the characteristics of an iron superoxide dismutase

(FeSOD). It was concluded that the increased activity of the FeSOD represented the initial activation of the antioxidant system in response to radical formation due to oxidation of SO_2.

3.6 Plant Resistance to Other Stresses

An open-air fumigation system was used to assess the development of mycoflora (yeast and filamentous fungi) populations on winter barley (*Hordeum vulgare* L.) cv. Igri in the presence of SO_2 or two fungicides (Magan and McLeod, 1991). Exposures (for the length of each growing season) to 37, 76 or 123 $\mu g\ m^{-3}$ (1986) or 37, 73 or 100 $\mu g\ m^{-3}$ (1987) caused a consistently lower number of colony forming units on flag leafs while the fungicides had no consistent effect. There was a significant decrease in pink yeast (*Sporobolomyces roseus*) populations in the high SO_2 plots for both years. White yeast (*Cryptococcus* spp.) populations also decreased with SO_2 exposure while populations of *Cladosporium* spp.were not significantly affected. Both fungicide treatments significantly reduced *Cladosporium* spp populations in 1986. A significantly greater percentage of green flag leaf area was found in the SO_2 treated plots compared to the fungicide treated plots. The authors indicated that yeast and *Cladosporium* species are an important component of cereal mycoflora and they can act as antagonists of foliar pathogens through competition for nutrients on the leaf surface. Thus, a reduction in these populations by SO_2 could reduce plant defences to pathogen attack.

Over a three-year period, Mansfield *et al.* (1991) examined the effects of SO_2 exposure in an open-air fumigation system on the development of fungal diseases of winter barley (*Hordeum vulgare* L.) cv. Igri. Sulphur dioxide concentrations of 24 (ambient), 55, 84 and 113 $\mu g\ m^{-3}$ were applied in 1984/85, 18 (ambient), 37, 76 and 126 $\mu g\ m^{-3}$ in 1985/86 and 13 (ambient), 64, 73 and 100 $\mu g\ m^{-3}$ in 1986/87, during the growing season. There was a general trend of increased powdery mildew infection (significant increases at some sample times) in plots exposed to SO_2, and decreased leaf blotch infection (significant decreases at some sample times). There was no effect of SO_2 treatment in any year on the incidence of sharp eyespot, *Fusarium* foot rot, brown rust, glume blotch, and net blotch on the flag leaves while variable effects were observed for eyespot and black ear moulds. The authors indicated that the variable response of disease to SO_2 could be a result of several interacting factors that have been shown to be affected by SO_2 including; host biochemistry, structure of wax layer, crop growth and growth of the pathogen.

The performance of the grain aphid (*Sitobion avenae* (F.)) on winter wheat (*Triticum aestivum* L.) cv. Rapier and winter barley (*Hordeum vulgare* L.) cv. Igri were assessed at varying levels of SO_2 exposure (in an open-air fumigation system) by Aminu-Kano *et al.* (1991) in a 3 year trial. Winter wheat was grown in the first year and winter barley for the final two years. The number of aphids on plants, plant sugar and plant nitrogen content were examined in treated and untreated plants. Aphid numbers on winter wheat treated with concentrations of SO_2 ranging from 63 to 149 $\mu g\ m^{-3}$ through the growing season increased linearly with increasing SO_2 exposure. Total nitrogen levels also increased with increasing SO_2 treatment and soluble nitrogen was significantly greater in all SO_2 treatments compared to the control. There was no effect of SO_2 on plant sugar content in wheat. In the first year of the winter barley exposure, the relationship between exposure (growing season average concentrations of 55 to 113 $\mu g\ m^{-3}$ SO_2)

and aphid numbers was very similar to the relationship observed in winter wheat; however, no effect of SO_2 treatment (seasonal means of 37 to 126 $\mu g\ m^{-3}$) was noted in the second year of the experiment. It was concluded that field exposures using low levels of SO_2 enhance aphid populations, probably through increased availability of soluble nitrogen in the plants (enhanced food quality for aphids).

3.7 Effects on Pollen

Bosac *et al.* (1993) studied the effects wet and dry exposure, both *in vivo* (on the anthers) and *in vitro* (culture dishes) on germination of pollen from oilseed rape (*Brassica napus*) cvs. Tapidor and Libravo. For in vivo treatments inflorescences were exposed in special chambers (excluding the rest of the plant) to 524 $\mu g\ m^{-3}$ SO_2 for 6 h. *In vitro* exposures (either wet or dry) were for 3 h. Exposure to 524 $\mu g\ m^{-3}$ SO_2 had no effect on germination or pollen tube length, *in vivo* or *in vitro* (dry); however, there was a significant reduction in germination when pollen was exposed to SO_2 while in unbuffered medium droplets. Pollen tube length was also greatly reduced under these conditions but too few pollen grains germinated and grew to calculate reliable means. It was concluded that the reduction in germination in the unbuffered medium was due to acidification of the medium (pH dropped form 6.5 to 5.5) during SO_2 exposure. Sulphur dioxide is highly soluble; therefore, would dissolve and acidify the medium during exposures.

Agrawal *et al.* (1995) utilized a nightshade (*Solanum nigrum*) complex, which exhibits three natural cytotypes [diploid (*S. americanum*), tetraploid (*S. villosum*), and hexaploid (*S. nigrum*)] to determine the effects of SO_2 on pollen chromosomes. Flowering plants were exposed to 524 $\mu g\ m^{-3}$ SO_2 for 2 h d^{-1} for 3, 7 or 11 days. When pollen mother cells (PMC's) were examined it was found that meiotic chromosomal abnormalities were highest in diploid plants (19.67 to 26.0%) and least in hexaploid plants (4.45 to 7.0%). In addition, abnormalities increased with length of exposure for all plants. Pollen sterility followed the same pattern as chromosomal abnormalities, 19.5 to 21.6% in diploid, 13 to 15% in tetraploid and 10 to 13% in hexaploid, with sterility increasing with length of exposure. The authors concluded that the observed abnormalities might have resulted from free radical splitting of phosphodiester linkages of DNA or from bisulphite combining with cytosine or uracil which may result in alteration of DNA or RNA functions.

3.8 Multiple Pollutant (Interaction) Experiments

Frost sensitivity in Scots pine (*Pinus sylvestris* L.) in response to SO_2 exposure, alone or in combination with NH_3, was investigated by Dueck *et al.* (1990). After a 5 month exposure in open-top chambers to 92 $\mu g\ m^{-3}$ SO_2, alone or in combination with 53 $\mu g\ m^{-3}$ NH_3, branches were frozen at -4, -7, or –10°C. Branches were then thawed, and electrolyte leakage (an indication of cellular damage due to freezing) was measured. No effect of SO_2 on frost hardiness was observed in branches exposed to -4 or -7°C. However, a significant increase in electrolyte leakage from SO_2-treated needles exposed to -10°C was observed compared to needles not

exposed to SO_2. The lack of an effect at -4 and -7°C was attributed to a low degree of frost hardiness in these plants. The authors suggested this might have resulted from the mild winter that preceded these experiments. In addition, the combination treatment resulted in a synergistic effect (more than additive) on electrolyte leakage in all freezing treatments. The combination treatment was the only treatment to cause needle injury, which was attributed to damage to the epicuticular wax layer.

Murray *et al.* (1992) examined the growth and yield responses of barley (*Hordeum vulgare* L.) cv. Schooner and subterranean clover (*Trifolium subterraneum*) cv. Trikkala to determine if co-exposure to NO_2 would alter the response to SO_2 alone. Plants were exposed for 108 days to SO_2 (<13 to 1,424 $\mu g\ m^{-3}$) and/or NO_2 (<10 to 337 $\mu g\ m^{-3}$) in open-top chambers for 4 h day^{-1}. Barley and clover had severe foliar injury at SO_2 concentrations of 686 $\mu g\ m^{-3}$ or higher, with no visible injury observed at the lower concentrations. Sulphur dioxide concentrations of 390 $\mu g\ m^{-3}$ or less produced no effect on vegetative shoot growth (dry weight) of barley while reductions of up to 70% were observed at 686 $\mu g\ m^{-3}$. Most growth parameters decreased linearly as SO_2 concentrations increased. When barley was exposed to SO_2 (144 or 390 $\mu g\ m^{-3}$) and NO_2 (337 $\mu g\ m^{-3}$), the number and weight of grain increased but protein content and foliar sulphur decreased. Exposure of subterranean clover to 390 $\mu g\ m^{-3}$ SO_2 resulted in a 30% increase in the longest branch length but higher concentrations resulted in sizeable reductions in branch length and above ground plant dry weight. When exposed to the SO_2 and NO_2 mixture, above ground plant dry weight and branch length were reduced. Shoot protein and shoot sulphur increased with increasing SO_2 exposure when exposed to SO_2 alone and to the mixture. It was concluded that barley and clover responded differently because of their differential access to nitrogen. Clover is a nitrogen fixer and likely has sufficient amounts of nitrogen compounds; thus, toxic levels of nitrogen derivatives could accumulate when exposed to NO_2.

Adaros *et al.* (1991a) used open-top chambers to examine interactive effects of SO_2, O_3 and NO_2, in two cultivars of spring barley (*Hordeum vulgare* L.) cvs. Arena and Alexis, and two cultivars of spring wheat (*Triticum aestivum* L.) cvs. Star and Turbo. Experiments were conducted over two growing seasons. In the first season (1988) plants were exposed to 56 $\mu g\ m^{-3}$ SO_2, 47 $\mu g\ m^{-3}$ O_3 and 55 $\mu g\ m^{-3}$ NO_2 singly, or in all combinations. During the second growing season (1989) exposures were 63, 121 and 60 $\mu g\ m^{-3}$ for SO_2, O_3 and NO_2, respectively. Sulphur dioxide exposures were continuous, while O_3 was for 8 h day^{-1} followed by NO_2 for 16 h day^{-1}. Significant decreases of 5 and 6% in the 1,000 seed weight were observed for barley cv. Arena in the 1st and 2nd seasons, respectively. Ear length exhibited a significant 3% increase in length; however, there was no significant effect on yield. No other effects of SO_2 were observed for this cultivar. The only significant effect observed in cv. Alexis was an 8% decrease in dry weight per 1,000 seeds in 1989. Significant interactions were observed for most of the parameters measured in the SO_2–NO_2 treatment for cv. Arena in 1988 but there were no significant interactions in 1989. There was little evidence of interactive effects in any of the treatments for cv. Alexis. Both wheat cultivars showed variable effect due to SO_2. In 1988 cv. Turbo had significant decreases in ear dry weight (8%), total plant dry weight (7%), grains/ear (4%), 1,000 seed weight (4%), and in grain yield (9%), while in 1989 the only significant decrease observed was a 5% decrease in the 1,000 seed weight. In contrast, the wheat cv. Star showed little effect of SO_2 in 1988 and a significant increase in yield (9%) in 1989. Plant weight and yield of both wheat cultivars were affected negatively in the SO_2–O_3 treatment in both years. The three-pollutant

combination generally had a negative effect on growth and yield in both years; however, there were no consistent significant effects of any of the pollutant mixtures. The authors indicated they were unable to adequately explain the differences in response for the two growing seasons.

In a similar set of experiments, Adaros *et al.* (1991b) exposed oil seed rape (*Brassica napus* L.) cv. Callypso for three growing seasons to SO_2, O_3 and NO_2 to determine growth and interactive effects. In the first growing season (1987) plants were exposed to SO_2 (46 and 88 $\mu g\ m^{-3}$) and O_3 (44 and 85 $\mu g\ m^{-3}$) singly, and in combination. In the final two growing seasons (1988 and 1989) exposures were the same as described above in Adaros *et al.* (1991a). In 1987, exposure to 46 $\mu g\ m^{-3}$ SO_2 resulted in small increases in some growth parameters but there was no effect on yield. Exposure to 88 $\mu g\ m^{-3}$ SO_2 negated these increases. For 1988 and 1989, there was also little effect of SO_2 on plants, only the number of seeds per pod was significantly increased (5%) in 1989. There were few significant interactions observed and they were not consistent over the 3 growing seasons. However, it was indicated that the few interactions observed were mostly antagonistic (eg. the positive growth in the presence of SO_2 was reduced or became negative when O_3 was added) and that interpreting interactive effects of low levels of air pollutants is difficult as plant responses are often highly variable.

In later experiments, Murray *et al.* (1994) studied the growth responses of barrel medic (*Medicago truncatula*) cv. Paraggio and alfalfa (*Medicago sativa*) to intermittent exposures of SO_2 and NO_2 to determine if the addition of NO_2 would alter the growth response to SO_2. Plants were exposed to 144 to 686 $\mu g\ m^{-3}$ SO_2 singly, or in combination with 320 $\mu g\ m^{-3}$ NO_2 for 4 h day^{-1}, 7 d $week^{-1}$ for 108 days. Sulphur dioxide significantly reduced stem length and shoot dry weight of barrel medic. In contrast, shoot sulphur content and digestible dry matter increased as SO_2 concentration increased. Addition of NO_2 further decreased stem length, shoot dry weight, and shoot nitrogen content. Significant interactions were observed at the highest SO_2 concentration and the results indicated an antagonistic interaction. For alfalfa, significant reductions were observed for stem length and shoot dry weight while digestible dry matter, shoot sulphur content, and shoot nitrogen content were increased. Sulphur accumulation was significantly reduced when NO_2 was added to the 390 and 686 $\mu g\ m^{-3}$ SO_2 treatments. Average leaf dry weight was not decreased at the highest SO_2 level in the presence of NO_2. This along with the reduction in sulphur accumulation suggested a protective effect of NO_2; however, addition of NO_2 generally resulted in greater growth reductions, for both species, than were observed in SO_2 treatments alone.

In another of the series of studies conducted as part of the Liphook Forest Fumigation Project, Holland *et al.* (1995) examined heights and basal diameters in three conifers: Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L. Karst.) and Sitka spruce (*Picea sitchensis* Bong. Carr.) in response to long-term (4 years) fumigation with 10, 34 and 58 $\mu g\ m^{-3}$ SO_2. Plants were also exposed to 1.3 times the ambient O_3 for a portion of each year but the authors indicated there was no evidence of any O_3 effects. No relationship was found between SO_2 and growth parameters for Scots pine (despite visual foliar injury). Growth of Sitka spruce was poor after 1988 (the second year of fumigation); however, this was attributed to nitrogen deficiency (information from another study at Liphook) and low rainfall during the second half of the experiment. The annual extension growth of leading shoots between October 1988 and 1990, of Sitka spruce was 38 and 93% greater than ambient controls in the 34 and 58 $\mu g\ m^{-3}$ SO_2

treatments, respectively. This growth increase was attributed to increased foliar nitrogen, a result of co-deposition of NH_3 with SO_2 (information from another study at Liphook). In contrast, from 1987 to 1989 Norway spruce showed decreases in stem basal diameter growth, which was related to SO_2 treatment

In a study investigating the effects of SO_2 and/or O_3 on foliar injury and stomatal leaf diffusive resistance Tripathi and Tripathi (1992) exposed rice (*Oryza sativa*) and white bean (*Phaseolus vulgaris*) plants in fumigation chambers to 524 $\mu g\ m^{-3}$ SO_2, 392 $\mu g\ m^{-3}$ O_3, or a combination of the two, for 6 h per day for 10 days. They found that visible injury first appeared on two-week-old seedlings of rice and bean after 2 days of exposure. By the end of the exposure period 20.8% (white bean) and 20.1% (rice) of the leaf area was injured. For white bean visible leaf injury was least in the combined SO_2 and O_3 mixture (16% of total area) while in rice the mixture resulted in the greatest amount of injury (28.6% of total area). They also observed a decrease in leaf diffusive resistance in both species when they were exposed to 524 $\mu g\ m^{-3}$ SO_2. Leaf diffusive resistance for both species increased, > 4-fold when plants were exposed a mixture of SO_2 and O_3 compared to SO_2 alone. It was concluded that the observed differences in leaf diffusive resistance and visible injury could not be explained on the basis of stomatal response alone as several morphological and physiological factors influence stomatal function.

In an attempt to correlate observed canopy damage of Norway spruce (*Picea abies* L. Karst.) with ambient SO_2, NO_2 and O_3, Slovik *et al.* (1996) used field data and modelling exercises. Measurements of the indicated gases were taken in six spruce stands in Germany and the annual averages were pooled. For the period of the study, the SO_2 concentration ranged from a high of approximately 50 $\mu g\ m^{-3}$ in 1985 to approximately 15 $\mu g\ m^{-3}$ in 1992. Nitrogen dioxide remained fairly constant and averaged between 19 and 24 $\mu g\ m^{-3}$ while the average ambient O_3 increased from 41 to 65 $\mu g\ m^{-3}$ during the study period. Damage was defined as "more than 25% needle loss" or "chlorosis of at least 60% of the canopy". Results indicated that there were no correlations between ambient NO_2 or O_3 and observed damage; however, from 1984 to 1989 there was a significant correlation between damage to spruce and ambient SO_2 (damage increased as ambient SO_2 increased). Possible interactions between the pollutants were not discussed.

Shaw *et al.* (1993) reported the effects of SO_2 (34 and 58 $\mu g\ m^{-3}$) exposure on needle necrosis in Scots pine (*Pinus sylvestris* L.) during the fumigation period of the Liphook Forest Fumigation Project. This study was initiated at the end of July 1988 as unusual foliar symptoms were noticed. Regression analysis indicated that the appearance of foliar injury was related to the mean SO_2 concentration during a critical growth period although injury did not become visible until 5 weeks later. This critical period when the majority of the trees were at the end of the bud bursting stage and needle elongation was about to start. At 58 $\mu g\ m^{-3}$ SO_2 a greater number of trees exhibited foliar injury in 2 of the 3 survey years and foliar injury appeared on the same trees in consecutive years suggesting that the sensitivity was genetic. A subsidiary fumigation chamber experiment was performed to see if the injury symptoms observed in the field could be duplicated. Concentrations of SO_2 (655, 1,310 and 2,619 $\mu g\ m^{-3}$ SO_2) for 4 hours on Scots pine seedlings produced no effects in any treatment. It was suggested that this may have been due to a low replicate number resulting in a few plants at the most sensitive stage of growth, and/ or low humidity during fumigation.

In another of the Liphook Forest Fumigation Project experiments, Peace *et al.* (1995) studied carbohydrate metabolism of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.). In Scots pine they found considerable decreases in soluble carbohydrates and activity of the regulatory enzyme, sucrose phosphate synthase, during the summer months in all treatments (34 and 58 $\mu g\ m^{-3}$ SO_2, with or without 1.3 times ambient O_3). Norway spruce showed a similar pattern of reductions but of a smaller magnitude. It was concluded that the observed reductions were a result of lower photosynthetic rates and CO_2 assimilation (data taken from other research measurements at the Liphook site).

Growth chambers were used to study the physiological effects of long-term exposure (7 weeks) to SO_2 (45 and 112 $\mu g\ m^{-3}$), NH_3 (64 $\mu g\ m^{-3}$) or a combination of the two (46 and 69 $\mu g\ m^{-3}$, respectively) on poplar (*Populus euramericana* L.) cv. Flevo (van Hove *et al*, 1991). Net CO_2 assimilation and stomatal conductance were reduced by 15% at 112 $\mu g\ m^{-3}$ SO_2 and the reduction in CO_2 assimilation was irreversible. It was concluded that the reduction in CO_2 assimilation was not a result of reduced activity of Photosystem II, but they could not speculate on the causative mechanism(s). Scanning electron microscopy indicated there was no injury to the leaf cuticle, stomatal complex or epidermal cells adjacent to the stomatal pores. No significant effects in measured parameters were observed following treatment with SO_2 and NH_3 together.

An investigation in the Liphook Forest Fumigation Project studied the effect of SO_2 and O_3, alone or in combination, on the levels of astringin and isorhapontin (antifungal components) produced in spruce bark (Pearce and McLeod, 1995). In addition, *in vitro* assays were used to determine resistance to the root- and butt-rot pathogen *Heterobasidion annosum* (Fr.) Bref. Sulphur dioxide exposure (34 or 58 $\mu g\ m^{-3}$, for 2 years) singly, or in combination with 1.3 times ambient ozone, did not affect concentrations of astringin and isorhapontin in Sitka spruce (*Picea sitchensis* Bong. Carr.) or Norway spruce (*Picea abies* (L.) Karst.) seedlings. An induced defence mechanism would require the use of energy reserves such as starch; therefore, starch reserves of the stem were assessed in branches from 3-year-old Sitka spruce. No significant differences were found between treatments. Susceptibility to infection by *H. annosum* was not affected by exposure to SO_2 and O_3 in either species.

3.9 Lichens

Bates *et al.* (1996) used a quantitative study of three lichen species (*Hypogymnia physodes* (L.) Nyl., *Lecanora conizaeoides* Nyl. ex Crombie, and *Evernia prunastri* (L.) Ach.) to determine the effects of pollutant treatments on colonization of three conifer species (Scots pine, *Pinus sylvestris* L.; Norway spruce, *Picea abies* (L.) Karst.; and Sitka spruce, *Picea sitchensis* (Bong.) Carr). Trees were exposed in a field fumigation system to 11 (ambient), 32, or 53 $\mu g\ m^{-3}$ SO_2 from May 1987 to December 1990, and ambient or 1.3 times ambient ozone from March-December 1988, May-December 1989, and February-December 1990. Observations of lichen abundance were performed 5-7 months after fumigation stopped. *Evernia prunastri* only colonized trees in plots with ambient SO_2 and was most abundant on Norway spruce. The

number of *H. physodes* was significantly reduced as SO_2 concentration increased, with the greatest numbers observed on Norway spruce. In contrast, the colonization of *L. conizaeoides* increased as SO_2 concentration increased, which was attributed to its tolerance or requirement for SO_2. Colonization by the three lichens was not influenced by the O_3 treatment.

Ranta (2001) conducted one of the more recent studies of lichen recolonization after ambient SO_2 levels had decreased from 160 $\mu g\ m^{-3}$ in 1973 to 2 $\mu g\ m^{-3}$ in 1999. Lichen diversity and percent cover were monitored once every 5 years from 1980 to 2000 at 25 sites in Tampere, Finland. Six reference sites away from the city centre were also monitored. In 1980, the study sites had an average of 0.7 species with 0.06% cover while the reference sites had a mean of 6.83 species with 7.79% cover. Recolonization was rapid after 1985, and by 2000 study sites had an average of 7.6 species with 10.91% cover and reference sites had an average of 13.83 species with 19.6% cover. The authors noted that there was no geographical pattern to the colonization but that recolonization of the city centre was probably due to the rapid decrease in SO_2 and the small size of the city.

Table 2 Summary of effects of SO_2 on vegetation

Note: When percent increases or decreases are given, these values are comparisons with controls unless indicated otherwise.

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Effects on Growth						
Agrawal and Verma (1997)	*Triticum aestivum* L. cv. M 206 and M 234 (wheat)	390 ± 20	4 h d^{-1}, 5 d wk^{-1}, 8 wks	Open-top chambers	Visible injury **Results below are for SO_2 + recommended nutrient treatment (lower nutrients resulted in greater injury):**	Greatest when no nutrients added
					Height	Reduced: 3.9% (M206), 4.8% (M234)
					Total biomass	Reduced: 10.0% (M206), 8.6% (M234)
					Total chlorophyll	Reduced: 20.0% (M206), 18.7% (M234)
					Ascorbic acid content	Reduced: 18.3% (M206), 16.2% (M234)
					Sulphate-S content	Increased significantly: both cvs.
					Yield	Reduced significantly: 13.3% (M206), 10.3% (M234)
Ashenden *et al.* (1996)	Native British species: *Agrostis capaillaris* *Anthoxanthum odoratum* *Arrhenatherum elatius* *Avena sativa*	262, with 2-h peaks of 524 twice per wk for 4 wk, then	Total of 83 d	Solardomes (small greenhouses)		
	Bromus erectus *Bromus sterili* *Cerastium fontanum* *Chenopodium album* *Dactylis glomerata*	262, with 3-h peaks of 786 3 times per wk for 3 wks, then				

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Ashenden *et al.* (1996) cont'd	*Dechampsia fleeuosa* *Desmazeria rigida* *Digitalis purpurea* *Epilobium hirsutum*	262 with 3-h peaks of 786, 5 times per wk for 3 wks				**Statistically significant responses:**
	Eriophorum vaginatum				Total dry weight	18 species average decrease of 43%
	Galium aparine				Root:shoot ratio	13 species average decrease of 36%
	Helianthus annuus				Total leaf area	7 species average decrease of 40%
	Hieracium pilosella				Leaf area ratio	20 species average increase of 45
	Helianthemum mummularium *Hordeum vulgare*					
	Leontodon hispidus					**Mean response of all 41 species:**
	Lolium perenne				Total dry weight	25% decrease
	Origanum vulgare				Root:shoot ratio	14% decrease
	Poa annua				Total Leaf area	10% decrease
	Poa trivialis *Urtica dioica* *Zea mays*				Leaf area ratio	23% increase
	Avenula pratensis *Brachypodium pinnatum* *Briza media* *Centaurea scabiosa* *Chamaenerion ngustifolium* *Conyza canadensis* *Dryas octopetala* *Festuca ovina* *Festuca rubra* *Holcus lanatus* *Koeleria macrantha* *Lotus corniculatus* *Plantago lanceolata* *Rumex acetosa* *Thymus precox*					No effects

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Clapperton and Parkinson (1990)	*Phleum pratense* (timothy)	80 (d avg. May to Sept.)	Emissions for 23 yrs prior to experiments	Field exposure Site 1 – 5 km downwind from source	VAM spore counts and VAM infection rate of roots of plants grown for 4 wks in soils from each site	714 spores/100 g DW soil Approx. 60% reduction (significant) VAM infection, relative to site 3
		27		Site 2 – 13 km downwind from source		872 spores/100 g DW soil approx. 24% reduction (significant) VAM infection, relative to site 3.
		<27		Site 3 – 20 km upwind (control)		1,372 spores/100 g DW soil.
Clarke and Murray (1990)	*Eucalyptus rudis* Endl. (eucalyptus)	132 (ambient <13)	8 h d^{-1}, 17 wks	Open top chambers	Average leaf area	Increased by 37% significant
					Average leaf dry weight	Increased by 29% significant
					Leaf sulphur content	Increase not significant.
		274			Height	Increase approx. 20% significant.
					Leaf abscission	Increased by 45% - significant
					Leaf sulphur content	Increased by up to 25% - significant
Coleman *et al.* (1990)	*Raphinus sativus x raphanistrum* (wild type radish) and *Raphinus sativus* cv. Cherry Belle (cultivated radish)	262, 629 and 1,048	24, 30 or 35 d	Growth chambers	Biomass production	Significant reduction in only one of five trials in Cherry Belle
					Variability in biomass production	Increased significantly as SO_2 concentration increased
Colls *et al.* (1992)	*Hordeum vulgare* L. cv. Igri (winter barley)	89 for 6 d, or 178 for 3 d, then ambient for 3 d, or 534 for 1 d, then ambient for 5 d	6 d cycle repeated 24 times over growing season	Open air fumigation system	Shoot dry weight, grain yield	No effect
Julkunen-Tiitto *et al.* (1995)	*Salix myrsinifolia* (willow), six clones	300	7 h d^{-1}, 5 d wk^{-1}, for 3 wk	Fumigation chambers	Salicin and chlorogenic acid	Significant reduction of 15% to 70%

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Salicortin, 2'-O-acetylsalicortin, other phenolics	No significant effect
					Soluble sugars in leaves (glucose, fructose, sucrose)	No significant effect
Kropff (1990)	*Vicia faba* L. (broad bean)	165 (1985)	Growing season	Open air fumigation	Total DW	Significantly increased 14 to 48%
					Total dry matter	Decreased by 17%
		74 (1988)			Yield (pods)	Decreased by 23%
					Total dry matter	Decreased by 9%
					Yield (pods)	Decreased by 10%
Murray and Wilson (1990)	*Hordeum vulgare* L. cv Schooner (barley)	110, 317, 670 and 1,354	4 h d^{-1} (light), 79 d	Open top chambers	Shoot height	Increased approx. 10% at 110 $\mu g\ m^{-3}$ SO_2
					Shoot weight	15% decrease at 317 $\mu g\ m^{-3}$ SO_2
					Shoot sulphur content	Linear increase: at 317 $\mu g\ m^{-3}$ SO_2 >2.5 fold over control
					Head weight and number	Decreased 45 and 20%, respectively at 317 $\mu g\ m^{-3}$ SO_2
Murray and Wilson (1991)	*Medicago truncatula* Gaerm. cv. Praraggio (barrel medic)	107 to 314	4 h d^{-1}, 7 d wk^{-1}, 72 d	Open top chambers	Shoot, stem, and leaf DW, shoot length, branch number, leaf number, flower number	No significant effects
		668 to 1,349			Shoot sulphur accumulation	Significant increase at 314 $\mu g\ m^{-3}$
					Shoot, stem, and leaf DW, flower number	Significant reductions (>40%)
					Shoot sulphur accumulation	Significant increase (approx 85%)
Qifu and Murray (1991)	*Solanum tuberosum* L. cv. Russet Burbank (potato)	288	4 h d^{-1}, 105 d	Top covered chambers		**Well watered plants (significant effects at final harvest):**
					Leaf area	20% decrease
					Leaf chlorophyll content	30 % decrease in 35 day old leaves
					Leaf sulphur content	> 100% increase
					All parameters measured	**Water stressed plants** no significant effects at final harvest

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Qifu and Murray (1991) cont'd		786				**Well watered plants (significant effects at final harvest):**
					Leaf area	40% decrease
					Leaf dry weight	25% decrease
					Tuber dry weight	35% decrease
					Leaf chlorophyll content	40% decrease in 35 day old leaves
					Leaf sulphur content	> 125% increase
						Water stressed plants (significant effects at final harvest):
					Leaf sulphur content	100% increase
					Other measured parameters	No effect
Qifu and Murray (1994)	*Glycine max* L. (soybean)	380	5 h d^{-1} for 3 wks followed by a 3-wk exposure to salinity stress	Top-covered chambers	Plant height, leaf number, and nodule number	No significant effects
					Leaf area	Decreased 33% - significant
					Chlorophyll content	Increased 19% - significant
					Shoot dry weight	Decreased 16% - significant
					Root dry weight	Decreased 25% - significant
					Shoot:root ratio	Increased 18% - significant
					Nodule fresh weight	Decreased 22% - significant
		786			Plant height, leaf number and nodule number	No significant effects
					Leaf area	Decreased 29% - significant
					Chlorophyll content	Increased 35% - significant
					Shoot dry weight	Decreased 16% - significant
					Root dry weight	Decreased 25% - significant
					Shoot:root ratio	Increased 23% - significant
					Nodule fresh weight	Decreased 20% - significant

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		380, 786	5 h d^{-1} for 3 wks preceded by a 3-wk exposure to salinity stress		Low salinity pre-treatment	Reduced or eliminated SO_2 effects on all growth variables
					High salinity pre-treatment	Severe injury, in high SO_2 plants died
SO_2 Uptake and Plant S Content						
Agrawal and Singh (2000)	*Bougainvillea spectabilis*, (Bougainvillea) *Cassia siamea* (Cassod tree), *Delonix regia* (Flame-tree), *Eucalyptus* hybrid (Eucalyptus), *Mangifera indica* (Mango), *Psidium guajava* (Guava)	49 - 233 (seasonal avg – readings once every 10 d for 24 h at 2 h intervals) 22 - reference site	3 yrs	Transplanted trees - field conditions along a gradient from 2 coal-fired power plants	Total foliar sulphur content	24, 35, 27, 33, 41 and 25% increase – species as listed at most polluted site compared to reference site
Manninen and Huttunen (1995)	*Pinus sylvestris* L. (Scots pine)	1980: 10 to 100 (mon mean), 56 to 1,700 (d mean) 1985: 8 to 61 (mon mean), 15 to 280 (d mean) 1989: 0 to 95 (m mean), 2 to 357 (d mean)	Long-term field exposures to SO_2 from a point source	Natural sites selected at different distances from the SO_2 source. Co-exposure to other pollutants (e.g., O_3)	Needle sulphur accumulation, cuticular damage	Seven and 19 month old needles - Significant correlation between ambient SO_2 concentrations expressed as monthly mean, or the sum of highest or second highest daily SO_2 concentrations of each month Needles harvested from sites with higher ambient SO_2 exposures exhibited a greater degree of cuticular damage

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Shaw and McLeod (1995) Liphook	*Picea sitchensis* Bong. Carr. (Sitka spruce), *Pinus sylvestris* L. (Scots pine), *Picea abies* (L.), Karst. (Norway spruce) (4 y old seedlings at start of treatments)	34 and 58	43 months (May 1987 to Dec 1990)	Open air field fumigation system	Foliar sulphur content	Increased in all species and at both SO_2 concentrations
					Foliar nitrogen content	Increased in both spruce species but not in the pine
		plus 1.3 x ambient ozone			Measured parameters	No effects
Visible Injury						
Clapperton and Reid (1994)	*Phleum pratense* (timothy - SO_2 tolerant and non-tolerant clones)	170	2 wks	Fumigation chambers	Visible injury	Experiment terminated at 1st signs of visible injury – chlorotic areas, brown and dead leaf tissue
					Shoot and root dry weight	Reduced compared to controls
Photosynthesis						
Darrall (1991)	*Hordeum vulgare* L. cv. Igri (winter barley)	Ambient, low, medium, & high (100, 113 or 126 are averages for high SO_2 tmt for each season)	3 growing seasons	Open-air fumigation system	Net photosynthesis, dark respiration, stomatal conductance, transpiration, internal CO_2 concentration	No consistent effects of SO_2 on any of the measured parameters
					Grain yield, plant total weight	No effect
Gerini *et al.* (1990)	*Zea mays* cv. Lenor G4441 (maize)	113	4 wks	Perspex chambers in a screenhouse	Photosynthetic activity	Reduced by 20%
		186				Reduced by 20%
		291				Reduced by 10%
		113			Quantum yield	Decreased significantly (>15%)

Reference	Species	SO_2 (µg m^{-3})	Exposure Duration	Experimental Method	Measurement	Result
		186				Decreased <5%
		291				No significant effect
		113			Light saturated assimilation rate	Decreased significantly (approx 20%)
		186				Decreased significantly (>15%)
		291				Decreased significantly (approx 10%)
		113			Transpiration	Increased significantly (approx 40%)
		186				Increased significantly (approx 30%)
		291				No significant effect
		113			Stomatal conductance	Increased significantly (approx 20%)
		186				Increased significantly (approx 15%)
		291				No significant effect (increased <5%)
		113			Water use efficiency	Decreased significantly (approx 40%)
		186				Decreased significantly (approx 35%)
		291				Decreased significantly (>15%)
		113			Intercellular/ambient CO_2	Increased significantly (approx 45%)
		186				Increased significantly (approx 35%)
		291				Increased <10%
Montiel-Canobra *et al.* (1991) Littlehampton	*Hordeum vulgare* L. cv. Igri (winter barley)	1985/86: 18 (ambient), 126	Growing season	Open air fumigation system	Flag leaf size (area and dry weight)	Significant reductions; area (20%) and weight (23%)
					Fructose-1,6-bisphosphatase activity	Significant reduction post-anthesis
					Phosphoribulokinase, NADP-dependent glyceraldehyde-phosphate dehydrogenase, phosphoglycerate kinase activity, chlorophyll content	No significant treatment effects
		1986/87: 13 (ambient), 73, 100			Flag leaf size (area and dry weight)	Significant reductions; area (20%) and weight (21%) at 100 µg m^{-3}

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Fructose-1,6-bisphosphatase activity	Significant reduction post-anthesis at 100 $\mu g\ m^{-3}$
					Phosphoribulokinase, NADP-dependent glyceraldehyde-phosphate dehydrogenase, phosphoglycerate kinase activity, chlorophyll content	No significant treatment effects
Lorenzini *et al*, (1995)	*Quercus cerris* L. (Turkey oak)	73, 160, 244	11 wks	Fumigation chambers	Photosynthetic activity	44% reduction (at 244 $\mu g\ m^{-3}\ SO_2$)
					Internal/ambient CO_2	15% increase (at 244 $\mu g\ m^{-3}\ SO_2$)
			23 wks		Foliar starch	15% increase (at 73 $\mu g\ m^{-3}\ SO_2$)
					Total S content	> 100% increase (at 73 $\mu g\ m^{-3}\ SO_2$)
					Plant height, leaf fresh weight	No effect
	Quercus pubescens Willd. (oak)	73, 160, 244	11 wks		Photosynthetic activity	5% reduction (at 73 $\mu g\ m^{-3}\ SO_2$)
					Water use efficiency	14% reduction (at 73 $\mu g\ m^{-3}\ SO_2$)
			23 wks		Foliar starch	10% increase (at 73 $\mu g\ m^{-3}\ SO_2$)
					Total S content	> 100% increase (at 73 $\mu g\ m^{-3}\ SO_2$)
					Plant height, leaf fresh weight	No effect
Odasz-Albrigtsen *et al.* (2000)	*Betula pubescens* (birch), *Vaccinium myrtillus* (bilberry), *Empetrumhermaphroditum Hagerup* (crowberry)	0 to 30 Avg ambient concentration from 1989 to 1992	Potentially since 1932 when the first smelter opened	Natural forest	Photosynthetic efficiency	Decreased as SO_2 concentrations increased
	Lichen species: *Hypogymnia physodes* L., *Parmelia olivacea Cladina* spp, *Parmelia olivacea*				Photosynthetic efficiency	Decreased as SO_2 concentrations increased

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	Pinus sylvestris L.(Scots pine), *Betula nana* L. (dwarf birch), *Arctostaphylos alpinus* L. (ptarmagin berry), *Salix lapponum* L. (lap willow), *Pleurozium schreberi* (Brid.) Mitt. (pine moss)				Photosynthetic efficiency	No significant effect
Panigrahi *et al.* (1992)	*Oryza sativa* L. cv. Jahati (rice) Plants 20, 40, 60, 80 and 100 d old *Phaseolus aureus* R. cv. Dhauli (mung bean) Plants 15, 30, 45, and 60 d old	655 1,310 2,620 5,240	6, 12, 24 and 48 h.	Fumigation system	Chlorophyll content	Decreased as SO_2 concentration increased, significant in all treatments Decreased as exposure duration increased in each SO_2 treatment
Ranieri *et al.* (1999)	*Hordeum vulgare* L., cvs Panda and Express (barley)	210	75 d	Perspex exposure chambers in a greenhouse	Leaf area, leaf, stem and root fresh weight	Significant reduction (data not shown)
					Photosynthetic activity	Reduced: Panda by 29%; Express by 49%
					Stomatal conductance	Decreased: Panda by 56%; Express by 58%
					Electron transport activity	Photosystem I - reduced: Panda by 7%; Express by 18% Photosystem II - reduced: Panda by 11%; Express by 24%
					Whole electron transport chain activity	Reduced: Panda by 27%; Express by 29%
					Chlorophyll *a*	Significantly decreased: Panda by 44%; Express by 10%
					Carotenoids	Significantly decreased: Panda by 46%; Express by 10%

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					ß-carotene content	Reduced: Panda by 48%; Express by 14%
					VAZ pigments (violaxanthin, antheraxanthin, zeaxanthin)	Reduced: Panda by 50%; Express by 15%
Veeranjaneyulu *et al.* (1991)	*Acer saccharum* Marsh. (sugar maple, 5-7 yr old)	131, 262, 1,310, 2,619, 5,238	4 h	Fumigation chamber	Quantum yield of O_2	Increased 65% at 131 $\mu g\ m^{-3}$ Decreased 50% at 5,238 $\mu g\ m^{-3}$
					Energy used for photosynthesis (PES)	Increased 24% at 131 $\mu g\ m^{-3}$ Decreased 22% at 5,238 $\mu g\ m^{-3}$
Other Metabolic Processes						
Bernardi *et al.* (2001)	*Phaseolus vulgaris* L. cv. Groffy (bean seedlings)	79, 157 or 236	2, 4 or 7 days	Greenhouse fumigation chambers	Visible injury.	None
					Newly synthesized polypeptides (34.5 and 31 kDa)	Observed in all treated plants.
					FeSOD activity	Induced at 79 $\mu g\ m^{-3}$ SO_2 2 d exposure
Borland and Lea (1991)	*Hordeum vulgare* L. cv. Igri (winter barley)	13 (ambient) 39, 73, & 100	Growing season	Open top chambers	Nitrate reductase activity	Significantly decreased at 100 $\mu g\ m^{-3}$ Feb., Mar., Apr.
					Nitrite reductase activity	Significantly increased at 100 $\mu g\ m^{-3}$ Apr. & May
					Glutamine synthetase activity, glutathione reductase activity, and total glutathione content	No significant effects
					Glutamate dehydrogenase activity	Significantly increased at 100 $\mu g\ m^{-3}$ Dec, Jan & Jun
Chauhan (1990)	*Lycopersicon esculentum* Mill. (tomato)	262	2 h d^{-1}, 15 d	Closed-top chambers	Ethylene emissions (percent increase over controls)	100%
		524	1 h d^{-1}, 15 d			100%
		262	2 h d^{-1}, 30 d			86.66%
Chauhan		524	1 h d^{-1}, 30 d			89.7%
(1990) cont'd		262	2 h d^{-1}, 45 d			64%

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		524	1 h d^{-1}, 45 d			81.65%
		262	2 h d^{-1}, 60 d			50%
		524	1 h d^{-1}, 60 d			71.42%
		262	2 h d^{-1}, 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d^{-1}, 15 d			Nil
		262	2 h d^{-1}, 30 d			100%
		524	1 h d^{-1}, 30 d			100%
		262	2 h d^{-1}, 45 d			100%
		524	1 h d^{-1}, 45 d			100%
		262	2 h d^{-1}, 60 d			88.88%
		524	1 h d^{-1}, 60 d			92.57%
		262 and 524	15, 30, 45 & 60 days		Acetaldehyde and Ethanol	100% increase over controls for both SO_2 concentrations and all exposure times
	Vigna radiata L. Wilczek (mung bean)	262	2 h d^{-1}, 15 d	Closed-top chambers	Ethylene emissions (percent increase over controls)	100%
		524	1 h d^{-1}, 15 d			100%
		262	2 h d^{-1}, 30 d			66.85%
		524	1 h d^{-1}, 30 d			77.47%
		262	2 h d^{-1}, 45 d			40%
		524	1 h d^{-1}, 45 d			52%
		262	2 h d^{-1}, 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d^{-1}, 15 d			Nil
		262	2 h d^{-1}, 30 d			100
		524	1 h d^{-1}, 30 d			100
		262	2 h d^{-1}, 45 d			75
		524	1 h d^{-1}, 45 d			80.39
Chauhan (1990) cont'd		262 and 524	15, 30, 45 d		Acetaldehyde and Ethanol	100% increase over controls for all exposure times

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	Zea mays L. (maize)	262	2 h d^{-1}, 15 d	Closed-top chambers	Ethylene emissions (percent increase over controls)	100
		524	1 h d^{-1}, 15 d			100
		262	2 h d^{-1}, 30 d			71.40
		524	1 h d^{-1}, 30 d			74.26
		262	2 h d^{-1}, 45 d			35.23
		524	1 h d^{-1}, 45 d			67.14
		262	2 h d^{-1}, 60 d			1.89
		524	1 h d^{-1}, 60 d			47.37
		262	2 h d^{-1}, 15 d		Ethane emissions (percent increase over controls)	Nil
		524	1 h d^{-1}, 15 d			Nil
		262	2 h d^{-1}, 30 d			Nil
		524	1 h d^{-1}, 30 d			Nil
		262	2 h d^{-1}, 45 d			100
		524	1 h d^{-1}, 45 d			100
		262	2 h d^{-1}, 60 d			64.28
		524	1 h d^{-1}, 60 d			67.46
		262 and 524	30, 45, 60 d		Acetaldehyde and Ethanol	100% increase over controls 30 to 60 d exposures
Gupta *et al.* (1991)	*Glycine max* L. Merr. (soybean)	131	1, 2, or 4 h	Plexiglas growth chambers	Visible injury	None
					ABA content of fully expanded leaves right after exposure	1 h – 28% higher than control (sig) 2 h – 87% higher than control (sig) 4 h – 141% higher than control (sig)
					ABA content after an 18 h recovery period	1 h – 23.6% higher than control 2 h – 17.2% higher than control 4 h – 42.8% higher than control
		524	1, 2 or 4 h		ABA content of fully expanded leaves right after exposure	1 h – 53% higher than control (sig) 2 h – 131% higher than control (sig) 4 h – 190% higher than control (sig)

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					ABA content after an 18 h recovery period	1 h – 50.5% higher than control 2 h – 57.5% higher than control 4 h – 46.7% higher than control
		1,048	1, 2 or 4 h	Plexiglas growth chambers	Visible injury	Leaf curl and necrotic areas visible within 4 h of start of treatment
					ABA content of fully expanded leaves right after exposure	1 h – 86% higher than control (sig) 2 h – 153% higher than control (sig) 4 h – 229% higher than control (sig)
					ABA content after an 18 h recovery period	1 h – 64.8% higher than control 2 h – 68.5% higher than control 4 h – 73.7% higher than control
Madamanchi and Alscher (1991)	*Pisum sativum* L. cvs. Progress and Nugget (pea)	2,095	210 min	Continuously stirred tank reactors	Total glutathione (ratio of exposed/control)	Progress - significant increase - 1.11 to 2.04 Nugget - increased - 1.42 to 1.69
					Reduced glutathione (ratio of exposed/control)	Progress - significant increase - 1.11 to 1.93 Nugget - increased - 1.37 to 1.59
					Glutathione reductase activity	Progress - significant increase of 35% Nugget – increased by 21%
					Superoxide dismutase activity	Progress - significant increase of 90% Nugget - no effect
					Ascorbic acid, oxidized glutathione content	No significant effects
Rao and Dubey (1990)	*Azadirachia indica* A. Juss (neem) *Mangifera indica* Linn. (mango) *Zizyphus mauritiana* Linn. (ber) *Syzygium cumini* L. Skeels. (jamun)	48 (monthly average)	Long-term field exposure. Study done for 12 months	Natural forest	Stomatal conductance	Decreased by 6 to 15%
					Sulphate content in leaves	Increased by 26 to 48%
					Leaf protein levels	Decreased by 11 to 35%

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Superoxide dismutase activity	Increased by 4 to 14%
					Peroxidase activity	Increased by 10 to 22%
		69			Stomatal conductance	Decreased by 12 to 21%
					Sulphate content in leaves	Increased by 41 to 61%
					Leaf protein levels	Decreased by 13 to 41%
					Superoxide dismutase activity	Increased by 9 to 14%
					Peroxidase activity	Increased by 15 to 24%
		79			Stomatal conductance	Decreased by 19 to 22%
					Sulphate content in leaves	Increased by 46 to 71%
					Leaf protein levels	Decreased by 20 to 43%
					Superoxide dismutase activity	Increased by 12 to 17%
					Peroxidase activity	Increased by 18 to 28%
		90			Stomatal conductance	Decreased by 26 to 28%
					Sulphate content in leaves	Increased by 65 to 92%
					Leaf protein levels	Decreased by 26 to 52%
					Superoxide dismutase activity	Increased by 16 to 20%
					Peroxidase activity	Increased by 23 to 33%
Plant Resistance to Other Stresses						
Aminu-Kano *et al.* (1991) Littlehampton	*Triticum aestivum* cv. Rapier (winter wheat)	1984: 29 (ambient) 63, 120, 149	Growing season (Nov to July)	Open air fumigation system	Aphid numbers	Significant increase with increased SO_2
					Plant nitrogen	Significant increase in soluble nitrogen
					Sugar content	No effects
	Hordeum vulgare cv. Igri (winter barley)	1985: 24 (ambient), 55, 84, 113 1986: 18 (ambient), 37, 76, 126	Growing season (Nov to July)	Open air fumigation system	Aphid numbers	1985: significant increase with increased SO_2 1986: no significant effects.

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Magan and McLeod (1991) Littlehampton	*Hordeum vulgare* L. cv. Igri (winter barley)	1986: 18 (ambient), 37, 76, 123 1987 13 (ambient), 37, 73, 100	Growing season (Nov to July)	Open air fumigation system	Pink yeast populations	Flag leaves: significant reductions, at highest treatment for both years Ears: significant reduction only in 1987 at highest treatment
					White yeast populations	Flag leaves: populations decreased Ears: same response as pink yeasts
					Cladosporium spp. populations	No consistent effect on populations on flag leaves or ears
Mansfield *et al.* (1991) Littlehampton	*Hordeum vulgare* L. cv. Igri (winter barley)	1984/85: 24 (ambient) 55, 84, 113 1985/86: 18 (ambient) 37, 76, 126 1986/87: 13 (ambient) 64, 73, 100	Growing season	Open air fumigation system	Powdery mildew	Increased infection with increasing SO_2 (significant increases at some sample times)
					Leaf blotch	Decreased infection with increasing SO_2 (significant decreases at some sample times)
					Eyespot, black ear moulds	Variable effects
					Brown rust, ear *Botrytis*, net blotch, *Fusarium* foot rot and sharp eyespot	No effect
Effects on Pollen						
Agrawal *et al.* (1995)	*Solanum nigrum* (Nightshade)	524	2h d^{-1} for 3, 7 or 11 d	Fumigation chamber	Chromosomal abnormalities	Diploid 19.67-26%, tetraploid 9-17.99%, hexaploid 4.45-7% of total
					Pollen sterility	Diploid 19.5-21.6%, tetraploid 13-15%, hexaploid 10-13% of total
Bosac *et al.* (1993)	*Brassica napus* L. cvs. Tapidor and Libravo (oilseed rape)	524	3 h	In vitro: petri dishes	Pollen germination and pollen tube growth – dry exposure	No effect

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Pollen germination and pollen tube growth – unbuffered medium	Significant reduction in germination. Tube length reduced (too few germinated to do statistics)
			6 h (pollen on anthers of intact plants)	In vivo: special chambers to only expose inflorescences	Pollen germination and pollen tube growth	No effect
Multiple Pollutant (Interaction) Experiments						
Adaros *et al.* (1991a)	*Hordeum vulgare* L. cvs. Arena and Alexis (barley)	56	108 d (1988)	Open-top field chambers	Straw, ear, plant dry weights, ears/pot, grains/ear, ear length, grain yield	No significant effects.
					1000 seed weight	Arena: significant 5% decrease Alexis: no effect
		Plus: 47 O_3, 55 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects
		63	99 d (1989)	Open-top chambers	Straw, ear, plant dry weights, ears/plant, grains/ear, grain yield	No significant effects
					Ear length	Arena: significant 3% increase Alexis: no effect
					1000 seed weight	Arena: significant 6% decrease Alexis: significant 8% decrease
		Plus: 121 O_3, 60 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects
Adaros *et al.* (1991a) cont'd	*Triticum aestivum* L. cvs. Turbo and Star (wheat)	56	108 d (1988)	Open-top chambers	Straw dry weight, ear length	No significant effects
					Ear dry weight	Turbo: significant 8% decrease Star: no effect
					Plant dry weight	Turbo: significant 7% decrease Star: no effect

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Ears/pot	Turbo: no effect Star: significant 9% increase
					Grains/ear	Turbo: significant 4% decrease Star: no effect
					1000 seed weight	Turbo: significant 4% decrease Star: significant 4% decrease
					Yield	Turbo: significant 9% decrease Star: no effect
		Plus: 47 O_3, 55 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects for the two growing seasons
		63	98 d (1989)	Open-top chambers	Straw, plant dry weights, grains/ear	No significant effect
					Ear dry weight	Turbo: no effect Star: significant 10% increase
					Ears/pot	Turbo: no effect Star: significant 8% increase
					Ear length	Turbo: significant 4% increase Star: no effect
					1000 seed weight	Turbo: significant 5% decrease Star: no effect
					Yield	Turbo: no effect Star: significant 9% increase
		Plus: 121 O_3, 60 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects for the two growing seasons
Adaros *et al.* (1991b)	*Brassica napus* L. cv. Callypso (spring rape)	46 or 88	89 d (1987)	Open top chambers	Dry weights of stems, pods, whole plant, and 1000 seed DW	At 46 $\mu g\ m^{-3}$ small increases (<15%) reduced to control levels at 88 $\mu g\ m^{-3}$
					Pods/plant and seeds/pod	Decreased from controls
					Yield	Significant 12% decrease at 88 $\mu g\ m^{-3}$
		Plus: 44 or 85 O_3			Measured parameters	No consistent significant interactive effects for the three growing seasons

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		56	113 d (1988)	Open top chambers	Dry weights of stems, pods and whole plant, number of pods per plant, seeds/pod, 1000 seed weight, and total yield	No significant effects
		Plus: 47 O_3, 55 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects for the two growing seasons
		63	84 d	Open top chambers	Dry weight of stems, pods and whole plant, number of pods per plant, pod length, 1000 seed weight, total yield	No effects
					Seeds per pod	Significant 5% increase
		Plus: 121 O_3, 60 NO_2 in all combinations	O_3 - 8 h d^{-1} NO_2 - 16 h d^{-1}		All measured parameters	No consistent significant interactive effects for the two growing seasons
Dueck *et al.* (1990)	*Pinus sylvestris* L. (Scots pine)	92	5 months	Open top chambers	Frost sensitivity as indicated by electrolyte leakage at -4, -7 or -10°C	Significant increase at -10°C.
		92 SO_2 + 53 NH_3			Frost sensitivity as indicated by electrolyte leakage at -4, -7 or -10°C	Significant increase at all 3 temperatures
					Visible injury	Needle tips brown (approx. 1/2 of needle)
Holland *et al.* (1995) Liphook	*Picea sitchensis* Bong. Carr. (Sitka spruce) (4-yr-old seedlings at start)	10 (ambient control), 34 and 58	43 months (May 1987 to Dec 1990)	Open air fumigation system	Estimated extension growth of leading shoots	Increased 38 and 93% in low and high treatments, respectively (regression significant)
	Pinus sylvestris L. (Scots pine - 4-yr-old seedlings at start)				Basal diameter growth, shoot elongation growth, height	No significant effect
	Picea abies L. Karst. (Norway spruce - 4-yr-old seedlings at start)				Basal diameter growth	Decreased at both treatment concentrations for 1988 and 1989 (regression significant)
					Height and estimated extension growth	No effect

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		Plus 1.3 x ambient O_3 (approx 30 $\mu g\ m^{-3}$)			Measured growth parameters	No effect
Murray *et al.* (1992)	*Hordeum vulgare* L. cv Schooner (barley)	13, 144, 390, 686 and 1,424	4 h d^{-1}, 108 d	Open top chambers		**Results shown for barley are for 144 $\mu g\ m^{-3}$ treatment**
					Foliar injury	None
					Vegetative shoot dry weight	Decreased by >10%
					Above ground biomass	Decreased by 15%
					Ear number per plant	Decreased by 10%
					Ear DW per plant	Decreased by 15%
					Grain number per plant	Decreased by 15%
					Grain weight per plant	Decreased by 25%
					Average kernel weight	Decreased by 10%
					Leaf sulphur content	Increased by >45%
					Grain protein content.	Decreased by 5%
		Plus: 337 NO_2			Ear dry weight, number and weight of grain per plant	Increased at 144 $\mu g\ m^{-3}$ SO_2, then decreased as concentrations increased
					Grain protein content	25% decrease at 144 or 390 $\mu g\ m^{-3}$ SO_2
					Leaf sulphur content	No effect at 144 $\mu g\ m^{-3}$ SO_2, then increased as concentrations increased
	Trifolium subteranneum cv Trikkala (clover)	144	4 h d^{-1}, 108 d	Open top chambers	Longest branch length	Increased by 20%
					Total sulphur content	Increased by 55%
					Protein content	Decreased by 10%
		390			Longest branch length	Increased by 30%
					Total above ground biomass	Decreased by 20%
					Total sulphur content	Increased by 100%
		686, 1,425			Longest branch length,	Decreased by >40%
					Visible injury	Severe

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
					Total above ground biomass	>75% decrease
					Protein content	Increased by >15%
		Plus: 337 NO_2			Longest branch length	Approx. 20% decrease at 144 or 390 $\mu g\ m^{-3}$ SO_2, no effect at higher levels
					Total above ground biomass	>50% decrease at 144 or 390 $\mu g\ m^{-3}$ SO_2, no effect at higher levels
					Sulphur or protein content	No effect
Murray *et al.* (1994)	*Medicago turncatula* cv. Paraggio (barrel medic)	144, 390, 686	4 h d^{-1}, 7 d wk^{-1} 108 d	Open-top chambers	Stem length	Significant reduction in 2 highest treatments
					Shoot nitrogen content	Significant increase in highest treatment
					Shoot dry weight	Significant linear reduction
					Digestible dry matter, S content	Significant increase
		Plus 320 $\mu g\ m^{-3}$ NO_2			Shoot dry weight	Decreased about 50% in 144 and 390 $\mu g\ m^{-3}$ SO_2 treatments
					Shoot nitrogen, S content	At 686 $\mu g\ m^{-3}$ SO_2, accumulation was reduced below that found in SO_2 treatment alone
	Medicago sativa cv. Siriver (lucerne)	144, 390, 686			Stem length, shoot dry weight	Significant decreases at 686 $\mu g\ m^{-3}$ SO_2
					Leaf dry weight	Significant decrease at 2 highest SO_2 levels
					Shoot S and nitrogen content	Significant increase at 2 highest SO_2 levels
					Digestible dry matter	Significant increase at 686 $\mu g\ m^{-3}$ SO_2
		Plus 320 $\mu g\ m^{-3}$ NO_2			Leaf dry weight	No reduction with increasing SO_2
					S content, shoot dry weight, digestible dry matter	Decreased in comparison to SO_2 treatment alone

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
Peace *et al.* (1995) Liphook	*Pinus sylvestris* L. (Scots pine seedlings)	34, 58	43 months (May 1987 to Dec 1990)	Open air fumigation system	Activity of sucrose phosphate synthase	Significant reduction (>40%) in summer
					Soluble sugars	Significant reduction (>55%) in summer
		Plus 1.3 x ambient O_3			Activity of sucrose phosphate synthase and soluble sugar content	No additional effects compared to SO_2 alone
	Picea abies L. Karst. (Norway spruce seedlings)	34, 58			Activity of sucrose phosphate synthase	<20% reduction in summer
					Soluble sugars	>10% reduction in summer
		Plus 1.3 x ambient O_3			Activity of sucrose phosphate synthase	No apparent interaction with SO_2
					Soluble sugars	Significant reduction (>55%) in July
Pearce and McLeod (1995) Liphook	*Picea sitchensis* Bong. Carr. (Sitka spruce) *Picea abies* L. Karst. (Norway spruce seedlings)	34 and 58	2 yr	Open air fumigation system	Bark concentrations of astringin and isorhapontin (natural antifungal agents)	No significant effect
					In vitro infection by *Heterobasidion annosum* (Fr.) Bref.	No significant effects
					Starch content – Sitka spruce	No effect
		Plus 1.3 x ambient O_3	1 yr		Measured parameters	No interactive effects
Shaw *et al.* (1993) Liphook	*Pinus sylvestris* L. (Scots pine)	34	43 months, May 1987 to Dec 1990	Open air fumigation system	Number of trees showing needle necrosis	1988: 7 (2 in ambient control) 1989: 5 1990: 8 (1 in ambient control)
		58			Number of trees showing needle necrosis	1988: 34 1989: 7 1990: 33
		Plus 1.3 x ambient O_3				No effects

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
	Pinus sylvestris L. (Scots pine - 4 yrs old at start)	655, 1310 or 2,619	4 h	Perspex growth chamber	Needle necrosis	No effect.
Slovik *et al.* (1996)	*Picea abies* (Norway spruce)	Ambient levels, 1984-1992 annual avg, approx. 15 to 50	Measurements on mature trees	Selected natural forest monitoring sites in Germany	Damaged canopy - needle loss and chlorosis	Significant correlation between damage and SO_2 levels between 1984 and 1989.
		19-24 NO_2 41-65 O_3				No correlation with damage
Tripathi and Tripathi (1992)	*Phaseolus vulgaris* cv. Pusa parvati (white bean)	524	6 h d^{-1}, 10 d	Fumigation chambers	Foliar injury	20.8% of leaf area injured
					Leaf diffusive resistance	Decreased slightly
		Plus 392 O_3			Foliar injury	16% of leaf area injured
					Leaf diffusive resistance	>4 fold increase over SO_2 treatment alone
	Oryza sativa cv. Saket-4 (rice)	524			Foliar injury	20.1% of leaf area injured
					Leaf diffusive resistance	Decreased slightly
		Plus 392 O_3			Foliar injury	28.6% of leaf area injured
					Leaf diffusive resistance	>5 fold increase over SO_2 treatment alone
van Hove *et al.* (1991)	*Populus euramericana* L. cv. Flevo (poplar)	45	7 wks	Fumigation chambers inserted into growth chambers	Net CO_2 assimilation, chlorophyll fluorescence, stomatal conductance, photochemical efficiency, quantum yield, cuticle integrity	No effect.
		112			Net CO_2 assimilation, stomatal conductance	15% reduction from controls
					Chlorophyll fluorescence, photochemical efficiency, quantum yield, cuticle integrity	No effects

Reference	Species	SO_2 ($\mu g\ m^{-3}$)	Exposure Duration	Experimental Method	Measurement	Result
		46 SO_2 plus 69 $\mu g\ m^{-3}$ NH_3			Net CO_2 assimilation, chlorophyll fluorescence, stomatal conductance, photochemical efficiency, quantum yield, cuticle integrity	No interactive effects
Lichens						
Bates *et al.* (1996)	Epiphytic lichens - *Hypogymnia physodes* L. Nyl., *Lecanora conizaeoides* Nyl. ex Crombie, and *Evernia prunastri* L. Ach	11 (ambient), 32 or 53	May 1987 to Dec 1990	Field fumigation	Colonization of young trees quantified 5-7 months after fumigation	*E. prunastri* only in ambient SO_2 *H. physodes* significantly reduced as SO_2 increased *L. conizaeoides* increased as SO_2 increased
		Plus 1.3 x ambient O_3				No additional effects

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